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ASSESSMENT OF THE BIOLOGICAL CONDITION OF THE NEW FORK RIVER, IN THE VICINITY OF THE PINEDALE ANTICLINE PROJECT AREA: 2010

AN ASSESSMENT OF CHANGES AMONG BENTHIC MACROINVERTEBRATE ASSEMBLAGES

PREPARED FOR:

Sublette County Conservation District
P.O. Box 647
1625 West Pine Street
Pinedale, WY82941

PREPARED BY:

Brett Marshall, MSc
River Continuum Concepts
P.O. Box 54
Manhattan, MT 59741

brett@RiverContinuum.org
http://www.RiverContinuum.org

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EXECUTIVE SUMMARY

STATEMENT OF PURPOSE

After the 2009 Task Group meeting, it became clear to me that, for some people, I had not made sufficiently clear the purpose of this study. Many federal and state agencies engage in habitat assessment and their familiarity with certain terms caused some confusion. Perhaps, the most confusing thing for some readers is that some of the terms used in benthic ecology are similar to terms used in geomorphology (cobble, substrata size, and sedimentation) and "habitat assessment." It is not the purpose of this biological survey to serve as a geomorphology or hydrology survey, nor a basic habitat assessment. This survey's purpose has always been to document the river's benthic ecology in such a way that the cumulative effect of both physical and chemical stressors could be documented in an efficient integrated manner; measurements are made at a scale appropriate for the response variables (aspects of macroinvertebrate community structure), whereas for hydrology surveys, direct measurements of the physical environment are in and of themselves response variables measured at the scale appropriate for larger scale assessments (spatial and temporal).

Apparently some readers had assumed that the purpose of our ecological survey was to replace a geomorphology study by using macroinvertebrates as a surrogate measure for habitat assessment. Although I do not know how widespread this misconception has become, I concur with those readers that the approach would make little sense. Therefore I want to reiterate the purpose very clearly here: *the purpose of this ecological survey is to document the composition of macroinvertebrate assemblages of the New Fork River in such a way that potential ecological perturbations can be assessed with some insight of cause and effect.*

PROJECT HISTORY SUMMARY

This report follows up eleven years of evaluation of the ecological condition of the New Fork River within the Pinedale Anticline Project Area (PAPA) using aquatic invertebrate assemblages. In previous years, we have identified some spatially-localized alterations of community structure which may be related to natural gas development in the PAPA. Ultimately, all the ecological descriptors indicating significant changes all reflected one underling community-level response: a dramatic and sustained elevation of the relative abundance (and by extension, relative importance) of the oligochate worms of the genus *Nais*. The effect was primarily localized to one of the nine study sites, below a natural gas pipeline, and existed from 2004-2008. This report details the findings from the 2010 field survey.

Nais are usually associated with organic deposits in fine sediments and changes in their abundance can be related to changes in the quantity or quality of fine sediment deposits. The results of past surveys indicated that there were active sources of erosion between NF30 and NF40 and that these were significantly correlated with changes in the abundance of macroinvertebrate fauna. Additionally, there were two factors that indicated that development of the PAPA may have been related to these changes. First, the pipeline capacity was increased annually by adding additional pipes across the New Fork River. Although the additional pipelines were bored beneath the stream bed riparian activities associated with pipeline placement, may have resulted in localized disturbance of the hyporheic zone. Second, there has been an increase in the development of drilling platforms in riparian areas; where there is clearly hyporheic flow. These platforms may cause ecological perturbation of river ecosystems by affecting hyporheic exchange and sedimentation.

2009 was the first year in nearly a decade to experience a "normal" sustained high flow from mountainous snow-melt. Spring run-off is an important natural phenomenon for western rivers and typically results in the sorting and redistribution of organic and inorganic substrata. Nine years of below normal flows may have prevented natural processes from mediating the effects of development in the PAPA. The elevated flow reduced the relative abundance of midges and worms, while increasing the richness of EPT orders and EPT abundance. The net effect was dramatically improved conditions at all sites on the New Fork River, and no-net change at the East Fork River site.

2010 RESULTS SUMMARY

The field survey described by this report collected eight 1-sq. ft. samples from each of nine sites on the New Fork River and one reference on the East Fork River, (NF17). The invertebrates of these samples were identified in the laboratory and used to calculate metrics that are used to evaluate the ecosystem function.

Analysis of spatial trends did not identify any particular longitudinal shifts in community structure that could be associated with development in the PAPA. There was a significant correlation on midges and non-insects (mostly Nais) with a new field covariate that summarized the relative plant cover of each square foot sample. Particle size was not as explanatory as plant cover or near-substrata flow.

Analysis of changes since 2009 indicated that every site showed a decline in the ecological condition based on the net change in 7 ecological summary measures. One site, NF04 declined significantly in all 7 measures and had several measures with values outside the range of expected values for reference streams in Wyoming Basin Ecoregion. The single cause of these changes was once again related to dominance of non-insects, and midges—both of which were strongly correlated with plant cover.

Analysis of change since 2007 produced several metrics at some sites which suggested improved water quality and the net effect of these indicated that NF50, NF60, and NF70 improved more than they declined. However, NF04 showed declines in water quality in all seven temporal measures. This indicates that NF04 was under more ecological stress in 2010 than any time in the since the sampling program began.

All the metrics indicating ecological decline at NF04 were related to increases in midges and worms. For instance, the increase in collectors and dominance was caused by the increased relative abundance of midges and worms. Similarly, since collectors comprised over 90% of the community at NF04, scrapers were forced to comprise less than 10%.

It is important to note that these changes appear to be correlated to plant cover in 2010, and unless there is some reason that PAPA development could increase plant growth; these changes are most likely caused by some influence other than natural gas development in the PAPA. Most likely, It is related to spread and growth of mats of the nuisance alga *Didymosphenia geminata*. To ensure that PAPA developers are not unjustly blamed for the effects of these algae, the program should begin to quantify the abundance of epileptic biofilm mass so that it can be accounted for as a covariate in our analyses.

CONTENTS

PREAMBLE:

There has been a trend in science writing to favor the active voice over the passive voice—particularly among ecological journals. To some, this may seem informal, but it is generally more concise—allowing more information to be discussed in less space, while promoting greater comprehension. Usually when we prepare single-author papers, the singular first person pronoun, "I" is used extensively. However, this report is the product of much work by the Sublette County Conservation District (SCCD) and their stakeholders so I usually used the plural pronoun, "we" to acknowledge their contributions and insights to the project.

ACKNOWLEDGEMENTS:

This report is the product of much work by many persons. I would especially like to thank Kathy Raper, Darrell Walker and Sno Ann Engler for their work on this report, in the field and logistical support. Also the SCCD Board of Supervisors for their commitment, continued involvement and funding of the project.

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APPROVAL:

This report has been reviewed by the Sublette County Conservation District. The work constitutes the final deliverable for the project: **PAPA 2010 Biological Monitoring**.

District Representative Signature Date

Assessment of the Biological Condition of the New Fork River, in the Vicinity of the Pinedale Anticline Project Area: 2010

AN ASSESSMENT OF CHANGES AMONG BENTHIC MACROINVERTEBRATE ASSEMBLAGES

1.0 BACKGROUND

The purpose of this study is to characterize the biological condition of the New Fork River, Sublette County, Wyoming, and to assess impacts related to natural gas development on the New Fork River. This monitoring program has been active for eleven years and incorporates the Sublette County Conservation District's baseline biological monitoring of the New Fork River. Although the focus is on 2007-2010, this report draws upon eleven years of biological data (2000-2010) to describe changes in the ecological condition of the New Fork River.

This study is more complex than a typical bioassessment because it needs to be; complexity is required to discern natural gas related impacts from natural variation in a very dynamic river system. There are several other forms of human influence (e.g., construction, sewer discharge) in the drainage as well as natural influences (e.g., stream size, substrata composition, mineral springs etc.) from which potential impacts will need to be differentiated. Thus, this study is more complex than many biological monitoring programs in the state, but it is that way because it needs to be to fulfill its purpose.

In 2007 we introduced several new methods to the study, including new sites to help tease out the influence of the sediment laden East Fork River and an altogether new study reach to assess potential impacts of increasing development on the northern portion of the Mesa. Additionally, we introduced an improved sampling method to the study which allowed improved statistical analyses. This report's analyses concentrate on the three years of quantifiable, replicated sampling, while drawing upon 11 years of descriptive trends and changes among sites.

PURPOSE

After the 2009 Task Group meeting, it became clear to me that, for some people, I had not made sufficiently clear the purpose of this study. Many federal and state agencies engage in habitat assessment and their familiarity with certain terms caused some confusion. Perhaps, the most confusing thing for some readers is that some of the terms are similar to terms used in geomorphology (cobble, substrata size, and sedimentation). It is not the purpose of this biological survey to serve as a geomorphology or hydrology survey, nor a basic habitat assessment. This survey's purpose has always been to document the river's ecology in such a way that the cumulative effect of both physical and chemical stressors could be documented in a timely manner; measurements are made at a scale appropriate for the response variables (aspects of

macroinvertebrate community structure), whereas for hydrology surveys, direct measurements of the physical environment are in and of themselves response variables measured at the scale appropriate for larger scale assessments (spatial and temporal).

Apparently some readers had assumed that the purpose of our ecological survey was to replace a geomorphology study by using macroinvertebrates as a surrogate measure for habitat assessment. Although I do not know how widespread this misconception has become, I concur with those readers that the approach would make little sense. Therefore I want to reiterate the purpose very clearly here: *the purpose of this ecological survey is to document the composition of macroinvertebrate assemblages of the New Fork River in such a way that potential ecological perturbations can be assessed with some insight of cause and effect.*

It just so happens that in recent years, localized patchy build up of fine sediment, presumably entrained from hyporheic disturbance, has been the cause of shifts in the composition of macroinvertebrate communities of the lower study area. Thus recent reports have had to discuss the implications.

1.1 BIOLOGICAL MONITORING BACKGROUND

Assessment of the biological condition of surface waters has become a key element in the comprehensive monitoring of water quality in the United States and beyond. State and federal agencies have been refining the techniques for regional assessment for about two decades (e.g., Plafkin et al. 1989, Barbour et al. 1999). However, these "rapid bioassessment protocols" use regional models that are not appropriate to evaluate the site-specific concerns required to assess change in the New Fork River. Site-specific designs, like those we use to assess the New Fork River are not new; Ruth Patrick of the Academy of Natural Sciences began performing such surveys as early as the 1940's. The Academy still conducts detailed faunal surveys to assess the effects of complex perturbations on the ecological processes of streams and rivers across the USA. As computing power has advanced, so too have the methods used to assess change in ecological communities; more complex calculations and simulations are now feasible.

Invertebrates are the most commonly used animal assemblage[1] used to describe ecological changes in rivers. "Benthic" is an adjective implying association with the bottom of streams or lakes. The "macro" part of the name means that, for much of the animals' life cycle, they are large enough to be seen without a microscope (though microscopes are required to identify them). Invertebrates are animals without backbones. Thus, we are studies of benthic macroinvertebrates are specifically concerned with aquatic insects, mussels, snails, worms, crayfish, crustaceans, mites, leeches and similar organisms. The monitoring program does not use data from bacteria (they are micro-invertebrates) or fish (they are macro-vertebrates). These groups can also be used for biological monitoring, but their spatial temporal scales of response are not appropriate for the local scale of this project and would not allow impacts to be located.

Invertebrates are incredibly diverse and abundant. They are also critically important because they play critical roles in detrital food webs—including the breaking down of complex organic material—and in transferring energy to higher trophic[2] levels by serving as food sources. Together, these aspects make macroinvertebrate assemblages excellent indicators of the overall health—or condition—of any ecosystem:

- They are numerous enough to be effectively sampled.
- They are diverse enough to exhibit response signatures.
- They are important and relevant to all "higher" animals.
- They respond rapidly enough to provide early warnings of problems.

[1] Assemblages are collections of species living together.

[2] "Trophic structure" refers to the level of organisms in the food chain (or food webs) and specifically refers to their roles in processing organic matter and moving its energy to other groups of animals. For example, algae, algae eating invertebrates, predatory invertebrates and fish, might represent different **trophic levels** in a food web.

- Their response to disturbance is recognized as important by many agencies.

For these reasons, benthic macroinvertebrates are often used to assess the effects of human activities to streams and rivers. Thus they may be used to describe the impacts of development and to describe the effectiveness of restoration (or mitigation). This is the rationale behind this study.

1.2 REVIEW PREVIOUS PAPA SURFACE WATER BIOLOGICAL MONITORING RESULTS

The most significant findings of previous reports indicated that the New Fork River was experiencing a significant active erosion source below the pipeline crossing between NF40 and NF30 (Marshall 2008). The macroinvertebrate assemblages at the site downstream from the pipeline were dominated by sediment tolerant organisms—mostly midges and worms (*Nais*). Mayflies and stoneflies were not as common at the site as they should have been[3].

A tour of riverside PAPA development completed after the report (Marshall 2008) indicated that many of the erosion controls implemented at drilling sites had failed over time and new drilling platforms often lacked erosion controls altogether. Additionally, several drilling platforms had been built in the floodplain, over hyporheic stream channels where they are likely to induce sedimentation of the New Fork River through sub-surface flows. These findings were not in the report, but were presented to the PAPA task group in September 2008 and again in October 2009. Some Water Task Group members (WTG) were incredulous that we had this discussion, but they may have forgotten that there is about a 12-month delay between data collection and final analysis with these reports.

Although the site tour indicated that operators were not doing all they could to prevent sedimentation in the New Fork River, it had been unclear how much influence multiple drought years influenced retention of sandy sediments. We do know that the New Fork River has experienced dramatically reduced flows from low precipitation from 2001-2005. Although the severity of drought reduced in recent years, the river has not recently experienced “normal” levels of sustained high flow consummate with snow melt until the year considered in this report (Fig. 1.1).

2009 was the first year in nearly a decade to experience a "normal" sustained high flow from mountainous snow-melt. Spring run-off is an important natural phenomenon for western rivers and typically results in the sorting and redistribution of organic and inorganic substrata. Nine years of below normal flows may have prevented natural processes from mediating the effects of development in the PAPA. The elevated flow reduced the relative abundance of midges and worms, while increasing the richness of EPT orders and EPT abundance. The net effect was dramatically improved conditions at all sites on the New Fork River, and no-net change at the East Fork River site.

[3] The expectations were drawn from upstream sites and from the SCCD baseline monitoring dataset.

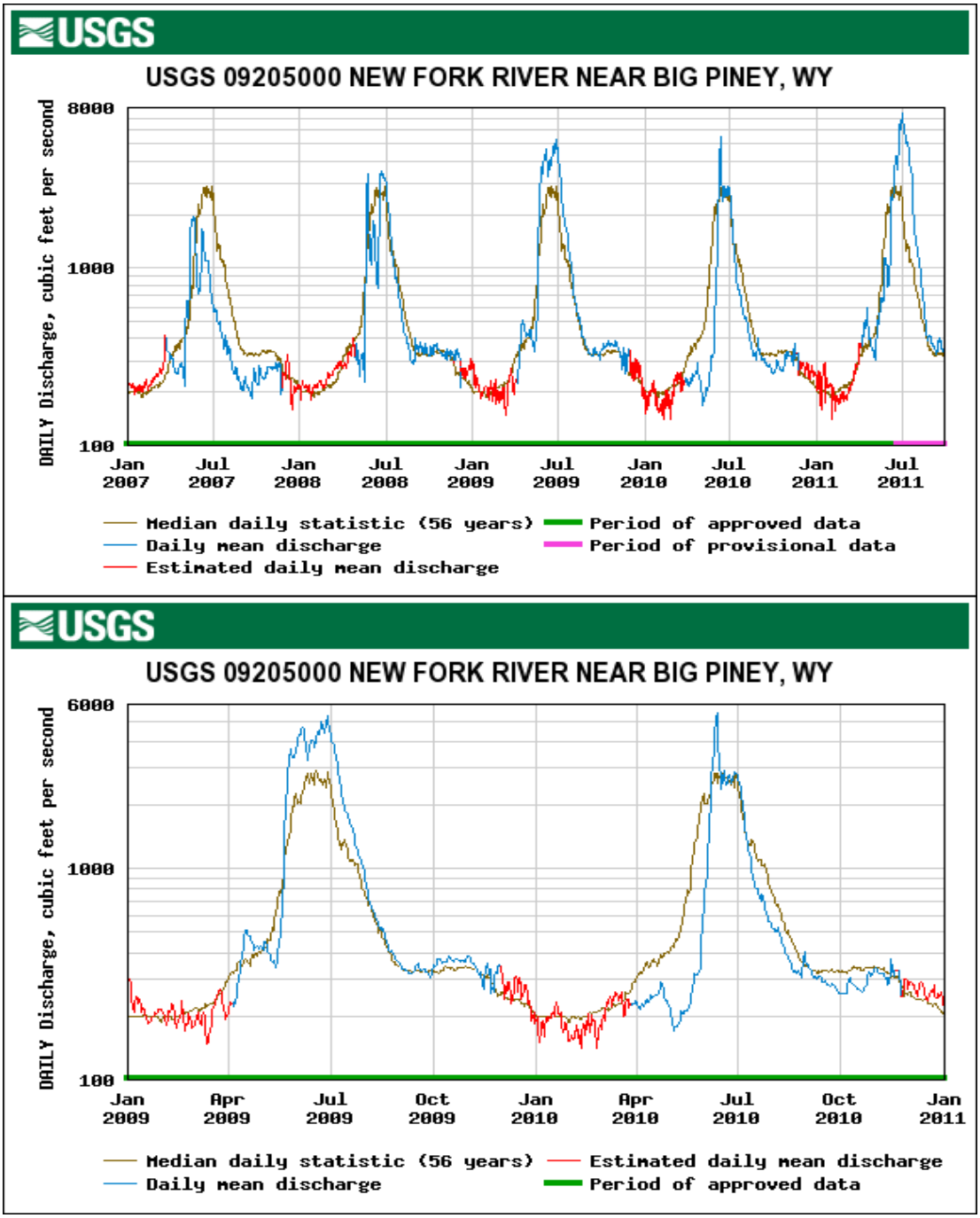


FIGURE 1.1. New Fork River Discharge 2007-2010. The upper figure highlights the below average flows of survey's early years. The lower figure shows 2009's sustained above average spring runoff pulse and the shorter duration of the 2010 spring pulse.

2.0 METHODS

2.1 STUDY SITES

Some of the sites used for this survey were part of the SCCD's baseline biological monitoring network for the New Fork River (NF01, NF04, NF17, NF19) and others were added later specifically to assess the influence of PAPA development on the New Fork River (NF30, NF40, NF50, NF60, NF70, NF80, NF90). In 2009, the site NF01 was replaced with a new upstream reference (NF80) because NF01 was too different (naturally) to serve as an adequate reference[4]. For this study, an additional upstream reference (NF90) was added because new development has encroached on NF80.

NF04 NEW FORK RIVER

NF04 is located south of Pinedale ~2 miles and is 50 feet downstream from the South Tyler Bridge. South Tyler is an access road for the PAPA. NF04, when established, was located upstream of the PAPA. A Wyoming Game and Fish Department fishing access and boat-launch are located at the sampling site. NF04 is also located downstream of the confluences of NF02 Willow Creek and NF03 Duck Creek; the confluence of these streams is believed to coincide with dramatic changes in the chemical and biological make up of the New Fork River (Marshall 2005a). Additionally, increased development on the north end of the Mesa may contribute potential runoff to the New Fork River upstream of this site.

NF17 EAST FORK RIVER

NF17 is located on the East Fork River, ~0.125 miles upstream of the confluence with the New Fork River. The Wyoming Game and Fish Department Boulder Fish Rearing Station is located upstream of NF17. NF17 is located downstream of HWY 191 approximately 5 miles. The East Fork River at NF17 is a sand dominated system with active sediment transportation occurring continually. In combination with several other sites, this site serves as a reference to account for changes downstream because it is a natural source of fine sediments that change the nature of the New Fork River's substrate composition and biology.

NF19 NEW FORK RIVER

NF19 is located on the New Fork River, upstream of the confluence with the Green River ~2 miles. The site is ~1½ miles downstream HWY 353. Badlands lie adjacent to the New Fork River upstream of NF19. NF19 is downstream from the PAPA. NF19 is the last sampling site in the New Fork River watershed. It serves to describe the condition of the New Fork River before it mixes with the Green River, and to help characterize the nature of upstream changes. Thus, this site is the ultimate

[4] NF01 exhibits about 50% of the discharge of NF80 and sites farther downstream. It is influenced by an impounded lake and is dominated by invertebrate species that do not normally occur downstream.

recovery zone site and we do not anticipate development in 2010 to reach this site. However, its continued status as a down stream reference could be compromised if gas development increases in the area.

NF30 NEW FORK RIVER

NF30 is located downstream of most of the PAPA development and below several pipelines' hyporheic crossings. The site is located on BLM land and has been sampled since the year 2001. A reclaimed gravel pit is located west of the sampling site. NF30 is located downstream of the confluence of the East Fork River (NF17) ~3 miles. Five replicated samples were collected at this site from 2004-2008. These samples represent the "study" community that was compared to NF40 and NF50 to describe the effects of development in the PAPA.

NF40 NEW FORK RIVER

NF40 is located within the PAPA and above the pipelines' crossings. The site is below the confluence of the East Fork River (NF17), Sand Springs and Alkali Draws and upstream from NF30 by about 1.5 miles. Five replicated samples were collected at this site during the years 2004 to 2008, but it was not sampled prior to 2004. These replicated samples originally represented the "control" community for comparisons with NF30 to describe the effects of the PAPA. The site is not an ideal control site because there is potential influence from Sand Springs Draw and Alkali Draw during runoff. This is likely to become more of a problem with planned development in the upper reaches of Sand Springs Draw. Thus, this site is now considered a measurement of the combined influence of Sand Springs and Alkali Draws, when compared to NF50.

NF50 NEW FORK RIVER

NF50 is located downstream of the confluence of the East Fork River (NF17) ~½ mile and upstream of Sand Springs and Alkali Draws. This site was established in 2007 to account for the effects of the East Fork River on the biota of the New Fork River. This is important because NF40 may be influenced by elevated sediment expulsion from Sand Springs and Alkali Draws. If this were to occur, there would be no way to differentiate the effect from the influence of the sand-laden East Fork River. A Bureau of Land Management public fishing access and boat launch area is located at this sampling site. Only biological data is collected at NF50 based upon the decision of the Pinedale Anticline Water Task Group. No chemical data is collected at NF50.

NF60 NEW FORK RIVER

NF60 is located upstream of the confluence of the East Fork River (NF17) with the New Fork River ~3/4 of a mile. NF60 was established in 2007 to describe the condition of the New Fork River before it is influenced by the East Fork River. This is important for documenting the influence of the East Fork River on the New Fork River at NF50. Only biological data is collected at NF60 based upon the decision of the Pinedale Anticline Water Task Group. No chemical data is collected at NF60.

NF70 NEW FORK RIVER

NF70 is located downstream of the confluence of Pole Creek ~¼ mile and downstream of NF04 ~ 4 miles. NF70 was established to monitor any effects from exploration and development from the northern portion of the Pinedale Anticline Project Area. This site measures the cumulative changes related to the gas development and the influence of Pinedale's sewage treatment plant (Pine Creek) which may change over time if facility management should change. Only biological data is collected at NF70 based upon the decision of the Pinedale Anticline Water Task Group (no chemical data available).

NF80 NEW FORK RIVER

NF80 is located downstream of the confluence of Duck Creek ~1 mile and upstream of NF4 ~1 mile. NF80 was established to monitor any effects from exploration and development from the upper portion of the PAPA. Upstream of NF80 is the town of Pinedale, a golf course and subdivisions. Both chemical and biological data are collected at this site.

NF90 NEW FORK RIVER

Site NF90 was added as the upstream control site for the surface water biological monitoring program when new exploration and development occurred upstream of NF80 in 2009. NF90 is located at a WYGF public fishing access and is known locally as "The Bull Pasture." It is a low gradient reach with benthic substrata dominated by aquatic vascular plants. NF90 is located north west of Pinedale approximately 5 miles and is upstream of the confluences of both Willow and Duck Creeks. Both biological and chemical data are collected at NF90. NF90 is the only site in Sublette County used as a reference stream for WY DEQ's Wyoming Stream Invertebrate Index.

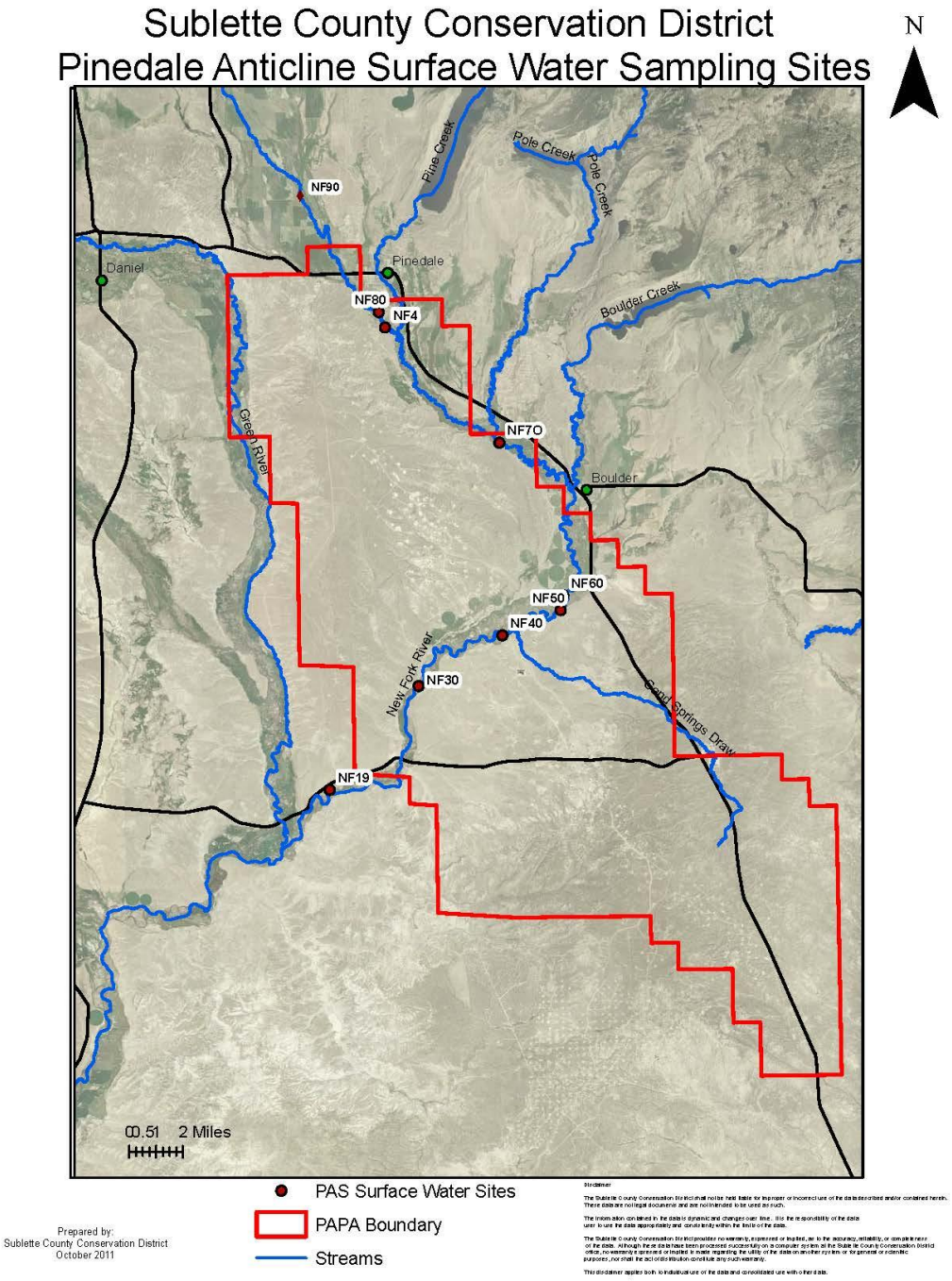


Figure 2.1. Pinedale Anticline Project Area. The study area consisted of nine sites from the New Fork River and one site on the East Fork River. The goal is to assess changes in the condition of the New Fork River as it passes though the Pinedale Anticline Project Area (outlined in red) of central Sublette County. Site NF01 is crossed out because it was replaced with site NF80 in 2008. NF90 was added as the upstream control site for the surface water monitoring program on when new exploration and development occurred upstream of NF80 in 2009.

2.2 HOW THE SITES FIT TOGETHER

The study sites represent a cumulative gradient of effects. Development of this study was a process of evolution from a simple comparison of two sites (NF30 and NF40 (Marshall 2005)) to a more complex study design using statistical procedures to tease out the effects of sources of variation not related to PAPA development. To understand how the sites fit together, we need to consider how the New Fork River might come to be influenced by development on the PAPA. We identified several modes whereby the integrity of the New Fork River could be affected by runoff from development on the PAPA. Currently there are two regions where potential effects of PAPA development are likely to accumulate as measureable impacts. We have separated these two areas into the upper and lower study areas to facilitate discussion and analysis. Graphs throughout the results section of this report have been bisected to clearly show the two study areas as well as the relative location of study sites along the downstream gradient. We have prioritized these locations based on the likely movement of surface waters during rain and snow melt events. This makes sense because these events are the most likely source of disturbance for surface waters—which are most likely to be in the form of eroded soil and sedimentation in streams. Additionally, if leachate or other industrial chemicals are spilled on soil, their eventual arrival in river systems is likely to correspond to runoff events. Note that there were no direct disposal effluents during the 2008 survey, but Anticline Disposal activated a permitted diffused, effluent during 2009.

Much of the development on the Mesa occurs in an area where the flow of run-off events is directed southeast, toward the lower study area. Similarly the development in the southeastern PAPA is most likely to experience runoff to the lower study area. In 2007, increasing concerns of increasing development in the northern PAPA necessitated a study area that could differentiate these influences from natural variation and anthropogenic influences farther upstream. Thus in 2007, the PAPA assessment added an upper study area.

THE UPPER STUDY AREA

Although most of the runoff from the Mesa flows southeast, there is an area on the northern edge of the Mesa which drains northerly. In 2007, three sites (NF01, NF04, and NF70) were sampled to account for changes in this study area. In 2008, NF01 was replaced with a more appropriate reference site (NF80) occurring downstream of Willow Creek and Duck Creek. In 2010, we added NF90, to ensure development near NF80 did not bias our upstream reference. We expect, NF80 and NF90 to reflect very similar ecological benthic communities unless development begins to degrade the ecological function of NF80.

Although the upper study area is smaller than the lower study area, the gradients are as complex as those occurring in the lower project area. Duck Creek and Willow Creek are known to influence the chemical and biological composition of the New Fork River (Marshall 2005a). These tributaries increase the conductivity of the New Fork River and seem to increase the amount of suspended and organic material.

NF70 integrates the effects of several smaller drainage systems off the Mesa, but is also influenced by Pine Creek and Pole Creek. These tributaries may dilute the waters contributed from Duck Creek and Willow Creek, but the influences of Pine Creek may change from year to year as it serves as a conduit for the Pinedale waste water treatment plant. This makes it difficult to assess PAPA-related influences from other anthropogenic stressors. Thus we added replicates to the NF70 site in 2007 so that statistical procedures could be used to correct for different sources of variation. Over the long-

term, the temporal changes occurring at this site relative to NF04 and NF60 (the upstream site of the lower study unit) will be important to diagnose changes within the upper study area and the lower study area as well.

The expected response signature for an extensive degradation of the New Fork River would include a decline in condition at NF80, or NF04, (relative to NF90) persisting farther downstream to NF70. Note that some ecological measures increase in response to ecological degradation, whereas others decrease.

THE LOWER STUDY AREA

Originally the primary concern was runoff directly from development on the southeast section of the PAPA. This was the rationale for the early addition of NF30, NF40 was added later to serve as a benchmark by which to gauge changes at this site. It soon became clear that this was not sufficient because we needed to account for changes from the New Fork River as well as potential impacts from runoff through Sand Springs and Alkali Draws, which enter the river downstream from the East Fork River and upstream from NF40. Thus several sites were added to account for this gradient.

NF17, on the East Fork River, had traditionally been represented by a single bioassessment sample, which did not allow us to account for variation in the New Fork River that may be related to inputs from this naturally sandy system. This site was recently augmented with replicate samples to allow us to include it as a spatial temporal variable in the statistical models. Conditions at NF50 should result from a combination of the conditions at NF60 and NF17. The difference between NF50 and NF40 may account for runoff flushed through the Sand Springs Draw and Alkali Draw. Direct runoff (as opposed to indirect runoff) from the PAPA would be represented from changes in the condition of NF40 to NF30 (Fig. 2.2).

Most of the land comprising the Mesa drains to the southeast. Thus, it appears likely that potential runoff and erosion could enter the river from the south-eastern edge of the Mesa. However, field investigations in 2006 indicated an extensive wetland system which would buffer the river from the effects of runoff from the southeast edge of the Mesa (Fig 2.2). Thus, the most likely source of impacts to the lower study area used to be runoff from the southeast portion of the PAPA —directly (i.e., pipelines or site-runoff to the northwest) or indirectly (via the draws). In 2007, development on the northwest side of the river encroached into the riparian zone—circumventing the natural wetland buffer. Now, the potential for impairment includes direct runoff and hyporheic disturbance from development on the northwest side of the lower study area.

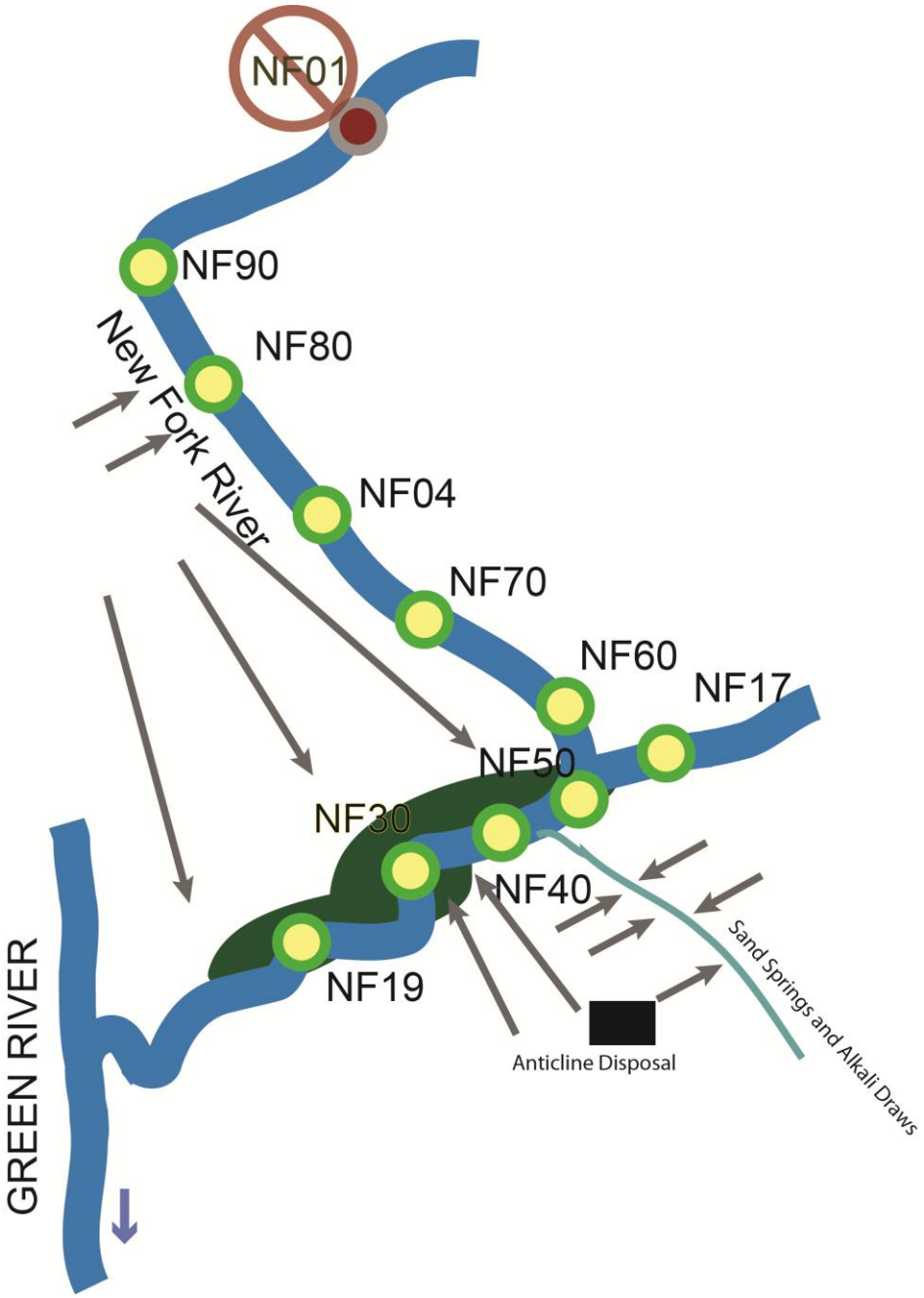


Figure 2.2. Study Site Schematic. This diagram shows the interrelationship among the location of study sites and potential sources of runoff in the PAPA. Arrows indicate potential vectors of influence on the New Fork River from development on the PAPA. Although most of the runoff on the Mesa drains to the southeast, this runoff encounters several wetland systems and is unlikely to actually reach the river. The sample sites are marked as circles on the river.

2.3 FIELD METHODS

In 2007, we altered the sampling plan to enable use of more substantial statistical analyses to differentiate anthropogenic changes in the New Fork River from natural variation—and variation not related to development on the PAPA. In the 2007 (Marshall 2008) report we reported both the historic method and the altered method simultaneously. This report primarily considers the augmented sampling method. This allows us to compare differences among sites in 2008 and to compare those sites with 2007 to evaluate change. This field method was called the "single Surber" (SS) method in earlier reports (Marshall 2008) and we retain that nomenclature this year.

SINGLE SAMPLE METHODS[5]

In 2007-2010, we collected eight single Surber samples from each site, each of which was processed individually in the laboratory to Wyoming Department of Environmental Quality (WY DEQ) standard procedures (e.g., Stribling et al. 2000). Collection procedures deviated from WY DEQ's standard methods, which were in pre-2007 PAPA assessments, in that WY DEQ usually "composites" all eight samples into a single sample representing the site in the field. For the SCCD field crew to collect replicates, they had to actually disturb 40 ft2 and remove all insects and debris from the bottom of the river. By keeping the samples separate, we can correlate them with environmental variables and increase the statistical power of assessment. Samples may be electronically composited at a latter time if necessary, but once they are composited in the field, this valuable variation is lost forever.

Single samples were collected using a stratified random sampling regime where near-substrate flow measures were used to ensure that the samples from each site fell within a uniform range of flows. This procedure is important for several reasons. First, it ensures that flows are uniform among sites. We know that near substrata flows can account for a very large amount of variation in aquatic invertebrate assemblages (e.g., Hart and Fonseca 1998, Hart and Finelli 1999) and we know that gas development is not likely to alter flow regimes. So by sampling consistent velocities we prevent this from producing confounding results.

In addition to ensuring flow consistency among velocities, sampling a range of flows at sites allows us to account for the effects of velocity on biological measures statistically. For this to succeed, each site needs to have a sufficient range of velocities to encompass a meaningful amount of biological variation, and we need the range to be similar among all sites[6]. This technique is called Analysis of

[5] In 2010, all samples were composed as single Surber samples, representing 1ft^2, with several replicates from each site. Prior to 2007, some sites in the PAPA were monitored using composite samples where each individual sample was composed of 8 Surber samples. The 2007 report found that there were significant differences in richness and diversity measures attained by the different methods, but that the single samples could be made comparable using rarefaction analysis.

[6] The target range included relatively slower riffle areas (~0.6fps) and faster riffle areas (~1.6fps).

Covariance (ANCOVA; Zar 1999) and it can be accomplished using the General Linear Models (GLM) algorithm (Wilkinson 2006) common among statistical software packages.

This technique also allowed us to relate other habitat variables directly to biological measurements. Many of these could be related to natural gas development—or due to natural variation. For example, the field crew measured the relative substrata size distribution, and embeddedness within the area defined by the Surber sampler for each sample.

2.4 LABORATORY METHODS

Biological metrics data from 2000-2004 were entered and validated by SCCD personnel and sent to Brett Marshall for analysis by a professional stream ecologist. Certified professional laboratories completed all the laboratory analyses and trained SCCD staff collected all field measurements. Thus, this report meets the requirements for credible data defined by the State of Wyoming. Most biological data from 2004-2008 were generated from raw taxonomic data by EcoAnalysts, Inc. Since this contract laboratory lost three samples in 2006 and 2008, we decided to process samples from 2009-2010 in-house at River Continuum Concepts.

Single samples (as discussed above), were subsampled to allow the identification of 200 organisms to the lowest practical taxonomic level; usually genus-species level. If the single samples contained fewer than 200 individuals, the entire sample was identified.

Previous laboratories used different levels of taxonomy and caused some artificial inflation of taxa richness. This occurred both within and among contract laboratories; it is part of dealing with large multi-species invertebrate data sets. Sometimes a taxonomist can place a solid species, species-group, or sub-species identity on one or two specimens, but can only identify some to genus level. When the data are compiled, this results in the same organism having three different names. We found this caused a problem in 2008's report (Marshall 2009). We used the same procedure that WY DEQ (Hargett and Zumberge 2006, Hawkins et al. 2008) and US EPA (Barbour et al 1999) have used to deal with this problem: we defined Operational Taxonomic Units (OTU[20]). Thus, all specimens were analyzed at a standard level of taxonomic effort--which truncates the dataset slightly to an appropriate level that is reproducible for all samples. The details of this process are documented in the Appendix to this report.

2.5 QUALITY ASSURANCE STATEMENT

The generally accepted norm for sorting efficiency (the proportion of the invertebrates actually removed for analysis) is that > 90% of the organisms in a sorted portion must be found and retained. The initial evaluation of the sorting efficiency indicated that although most samples were processed at >95% efficiency (average 94.4%), but that several samples failed to meet the same standard. One sample scored as low as 70% indicating that upon resorting, we found 30% of the organisms had been missed (Fig. 2.3).

We initiated a complete re-sort of the processed detritus of all samples at no cost to the clients. At the end of this process, all samples were brought up to the 90% standard, and most were at 100%. When the efficiencies of these re-sorts were evaluated, we found zero countable[7] organisms. Thus the final QA sort check indicated 100% efficiency.

The poor efficiency scores were due to one new technician, near the end of the project. However, the resorting process corrected any bias this would have caused to this survey. Most of the invertebrates missed by the technician were very small worms (*Nais* sp.) in samples with lots of algae or moss.

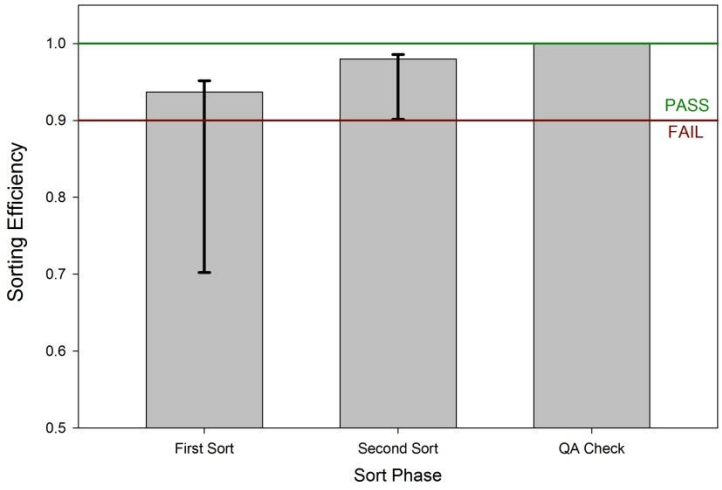


Figure 2.3. Sorting Efficiency. The final sorting efficiency of the samples used in this survey was ~100%, although initial sort included a few samples with unacceptably low scores. This was fixed using an additional sorting phase which effectively brought the efficiency to 100% at no cost to the clients. The top error-bars indicate 95% confidence intervals; the bottom error bar indicates the lowest single observed score.

[7] Note that small parts of insects and other invertebrates are considered uncountable if counting them could result in a single specimen being represented in the data set more than one time. For instance, many mayflies lose most of their legs in the samples. If mayfly legs were countable, the same insect could be tallied up to six times.

2.6 ANALYTICAL METHODS

PHYSICAL VARIABLES

The area contained within each SS benthic sample was described to provide sample-specific habitat data. These data were collected and recorded by SCCD during field collection and added to the analytical data set. These measures included depth, flow (6/10 depth and near substrata), % size composition of inorganic substrata (Wentworth 1922), embeddedness and plant cover (%).

In similar assessments (Marshall 1997, 1998, 1999, 2007a) conducted for the Academy of Natural Sciences, I have found that compiling substrate size distribution data into a Particle Size Index (PSI) has certain advantages. It correlates well with biological metrics and avoids problems with autocorrelation caused by using all the measures (which are proportional to each other). The index I have used in the past weighs the percentage of each substrate size class, relative to the suitability for invertebrate colonization. For example, many invertebrate species do not like sand—it moves in the river flow and could bury them or grind them up. Larger particles provide more stable, colonizable macroinvertebrate habitat. Optimal balance of providing surface area and stability is attained by the cobble-sized particles. Boulders are stable, but have less surface area per unit volume to accommodate diverse communities.

PSI= 0*fines + 1*Fine Gravel + 2*Coarse Gravel + 3*Pebble +4*Cobble + 1*Boulder

SCCD personnel visually estimated the relative portion of the sampler ($1ft^2$) that was covered with vascular plants and thick algae. The values were combined into a single measure of percent cover value by SCCD and used as the covariate PLANT in the GLM[8] statistical algorithm to account for the variation in metrics that could be accounted for by plant cover.

Embeddedness was measured as the amount of cobble buried by fines, measured in millimeters. The SCCD measured embeddedness three times and reported the average, which was used as the covariate EMBED in the GLM statistical algorithm to account for the variation in metrics that could be accounted for by embeddedness.

Flow was measured by SCCD in the field using two methods. Only the measurements taken near the river bottom were used to define the covariate FLOW, which was used in the GLM algorithm to account for variation in the metrics that could be accounted for by the velocity of water where each sample was collected.

[8] See description under "Statistical Analyses" below.

BIOLOGICAL VARIABLES

Biological metrics are values calculated from the taxonomic data set (which is a list of the species collected and their abundance) because they summarize the changes in species composition in terms of changes in ecological function. Metrics were used as the response variables for most analyses. This was necessary because the abundances of species change naturally though time and in space due to changes in the environment, inter-species competition and other factors. Ecological theory predicts that the functions performed by these species should be conserved—unless the ecosystem's function in impaired. That is, the abundance of each species may change naturally as a response to climatic variation or natural biological cycles, but usually a reduction in the abundance of one species is accompanied by an increase in the abundance of similar species. Thus, measures like the relative abundance of collector-gatherers should be more consistent than the abundance of individual species comprising the collector-gatherer guild. This is how metrics reduce the variability in species abundances by summarizing functional changes. The metrics compared in this report are discussed briefly below.

Taxa Richness is a very common metric that is used to describe the function of terrestrial and aquatic ecosystems. The measure is calculated by counting the number of different species (or similar kinds) in the sample. For aquatic ecology, the underlying philosophy is that more species can live in clean water than in polluted water. Therefore, higher values of Taxa Richness indicate a "healthier" condition and lower richness values may indicate an impaired condition.

The orders Ephemeroptera, Plecoptera and Trichoptera (mayflies, stoneflies and caddisflies, respectively - EPT) are generally considered to be more sensitive to disturbance than other organisms. Although not universally true, many of these organisms need cool, flowing water with high oxygen and low ion concentrations year-round. Thus, one of the most popular metrics in the United States today is the EPT index, which is the taxa richness of these three sensitive orders (e.g., Lenat and Penrose 1993). Because these orders do not always respond uniformly, many states—including Wyoming—have started using the richness of each of the EPT orders as separate metrics. Thus, three of the metrics used in the WSII are the richness of Ephemeroptera, Plecoptera and Trichoptera represented separately. We used combined EPT richness metrics to compare NF30 directly to NF40, but the richness of the individual EPT orders was used to calculate the WSII and compare samples to the regional reference condition.

The abundance of chironomid midges is often used as an indicator of environmental perturbation because there are 4000 species known from the northern hemisphere. Some of the common species are very tolerant to certain stressors and reach very high abundances when densities of predatory insects or competitors are reduced in polluted waters. This metric responds to organic enrichment and sedimentation. Specific taxa comprising the chironomid assemblage can be particularly useful for describing the causes of changes in multi-metric indices (like the WSII) and other metrics.

North American streams are normally dominated in abundance, richness, biomass, and production by aquatic insects. The notable exceptions are high-mineral springs and highly disturbed streams. Thus, high numbers of non-insect invertebrates often indicate that streams are stressed, or that there are unusual circumstances governing the community structure. Some non-insects, such as the ubiquitous amphipod Hyallela sp., are very tolerant of stress from high temperatures and elevated salinity. Others, like aquatic earthworms are tolerant to organic or inorganic sedimentation. Thus, specific taxa can be useful to help diagnose the causes or nature of anthropogenic perturbations.

There are five common macroinvertebrate functional feeding groups (FFG) used to classify taxa by their roles in processing organic material. The generalists eat fine particles of organic material that require little chewing to fit in their mouth. Their FFG designation is "collectors" and they are further subdivided into collector-gatherers and collector-filterers. Gatherers are deposit seekers whereas filterers remove fine particles from the water column. Predators are the FFG that preys upon other animals. Scrapers have adaptations to scrape fine layers of algae from rock and other hard surfaces. Shredders play very important roles in breaking up deposits of course detritus and woody debris--resulting in food availability for all other groups[9]. In streams with extreme sedimentation problems, extreme pollution, or even urbanization, the community tends to shift towards FFG composition dominated by collectors and predators.

STATISTICAL ANALYSES

The goal of this monitoring project has a different goal than comparing with other streams throughout Wyoming; we want to know if gas development in the Pinedale Anticline Project Area is changing the biology of the New Fork River. This is a much more complicated question than can be answered by the WSII's narrative condition criteria. We know from past experience that there are some natural deviations from the regional references of the WSII. However, our study design was developed to allow us to use the WSII to test sites NF30 and NF40 for changes related to the PAPA. Additionally, we use metrics that have historically been useful in the New Fork River (Marshall 2005a). We used SYSTAT v.12 statistical analysis software for most analyses.

We used Analysis of Variance (ANOVA; Zar 1999) to test for differences among sites in 2010, (treatment = SITE). The ANOVA used the within site averages and variance to determine the likelihood that the levels of each treatment are sufficiently similar to be considered statistically representative of the same population of data. In application, a P-value (probability) that is small means that there is a low probability that the observations are sufficiently similar to belong to the same "group." The convention among research scientists is to use a critical P-value of P=0.05 (5%) as the decision threshold. Thus, if P<0.05, there is >95% likelihood that the compared groups are not homologous. Another way to say this is that the probability of "type-1 statistical error" is less than 5%; we have a < 5% chance to incorrectly conclude that homologous groups are not actually homologous.

Although a very low type-1 statistical error is paramount for sound science, it has been criticized for environmental monitoring because it may cause real and important environmental changes to be obscured by natural variation. To avoid this conundrum, we also examined all metrics with a more-liberal P-value (P<0.10) and called these changes "marginally statistically significant" or "marginally significant". When these terms arise they mean that the result was not significant at the 95%-level, but was at the 90%.

[9] Shredders release nutrients bound in coarse particles to aid in algae production for scrapers, as well as making fine particles for collectors. By providing sustenance for these groups they also ensure prey is available for predators. If you are interested in FFGs and their role in community ecology, read the River Continuum Concept (Vannote et al. 1980), available at www.rivercontinuum.org.

When appropriate, Tukey's HSD or Fishers LSD tests were used to follow-up ANOVA results to determine which specific sites were significantly different from each other. These tests used the same critical p-values used for the ANOVA, allowing for significant differences if the probability of type-1 statistical error was less than 5%. We summarized these results using letters to define groups of sites that were not significantly different from each other. In tables and figures, each site is given a letter (or set of letters) and sites sharing a common letter in the results were not significantly different from each other. For example, a site marked as ACD and a site marked as BCE would not be considered different from each other because they share the letter C. However, a site identified by AC would be significantly different from a site marked B because they share no common letters based on the results of Tukey's or Fisher's tests.

This report follows the tradition set by the 2007 (Marshall 2008) and 2008 (Marshall 2009) reports and uses methods that allowed more efficient use of statistical analyses than in previous reports. Among these were methods that allowed us to include habitat measures in analyses of biological data. We used the General Linear Models (GLM) algorithm (available in most statistical software) and used metrics as response variables and all the habitat variables as predictors. The modeling procedure then removes predictor variables (i.e., flow, particle size, embeddedness etc.), which, when tested, do not explain a significant amount of variation in a specific response variable (i.e., metric). These are removed one at a time until only the variables that significantly explain variation in the model being developed and tested are retained. The process is complete when only variables that are significant—given the other variables in the model--are included[10]. For this reason the procedure is called a "backwards step-wise multiple regression modeling algorithm," but throughout this report we call it simply the "multiple regression." This procedure was especially useful to describe which metrics appeared to correlate with velocity or sedimentation.

[10] For example, a metric such as "%Filterers" might be strongly correlated with velocity and particle size. However once one of the variables is in the model, the contribution of the other may be non-significant, and that variable is excluded from the model. ALSO NOTE: "given the variables in the model" has a statistical meaning; it is not an incomplete sentence about giving something.

3.0 RESULTS

3.1 PHYSICAL MEASURES (COVARIATES)

The sampling regime for all other variables was based on selecting samples from a uniform flow range between sites. This was a very important aspect of the sampling plan because it allowed us to stratify the sampling plan without bias (a statistical concern) and allowed us to control an unwanted source of variation on the invertebrate community. That is, invertebrate assemblages are known to respond to water velocity, but we do not anticipate development on or near the PAPA to increase the velocity of water in the New Fork River. Thus, if we collected samples from the same approximate range of flows at all sites, we could control for this variation and account for it statistically. This constitutes a stratified sampling scheme that is superior both to completely randomized designs and to subjective stratified sampling methods.

As in 2008-2009, the SCCD field crew did an excellent job of sampling a consistent range of flows in 2010 and sampled the same average flow[11] as sampled in previous years. This is important because deviation in the average value would have been likely to confound analysis among years.

For 2010 there was no significant difference in flow regime sampled at the sites (P=0.804)[12].

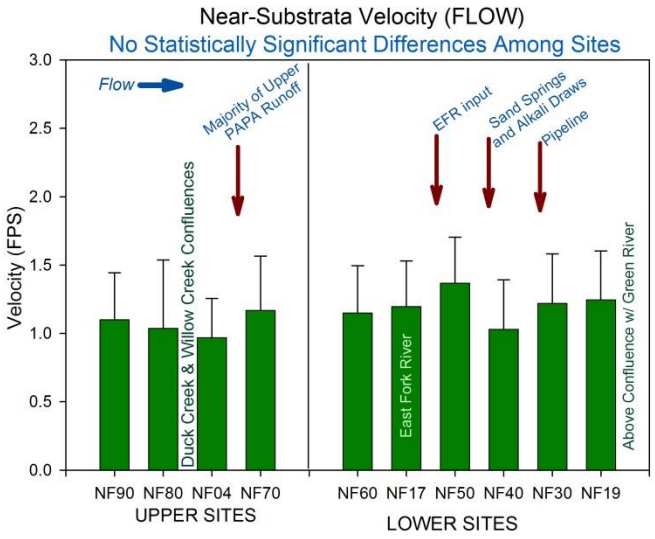


Figure 3.1. Near Substrata Flows. The average near-substrata water velocity from which benthic samples were collected is represented by bars. Sites are arranged from upstream to downstream. Error-bars represent 95% confidence interval of the mean.

[11] Recall that "flow," as used here, refers to velocity of water measured as close to the stream bottom as possible to serve as a covariate for benthic analysis.

[12] Recall there was a significant difference in 2007, but fortunately, there was no interaction effect in the Analysis of Covariance used to compare sites and adjust for flow (Marshall 2008)).

SUBSTRATA SIZE COMPOSITION

The size of particles[13] comprising the stream bottom is important for the success of macroinvertebrates as well as for fish reproduction. The field crew quantified the size composition substrata (Fig 3.2) within each Surber sample. Thus, these data do not describe the totality of particle sizes found at each site, but rather where the benthic samples were collected—as assorted[14] by New Fork River flows[15]. Since the effort was standardized by water velocity, the sites should be somewhat similar—unless something other than flow has influenced the distribution of particles in the river. For example, we know from field observations that smaller particles should naturally dominate the East Fork River (NF17).

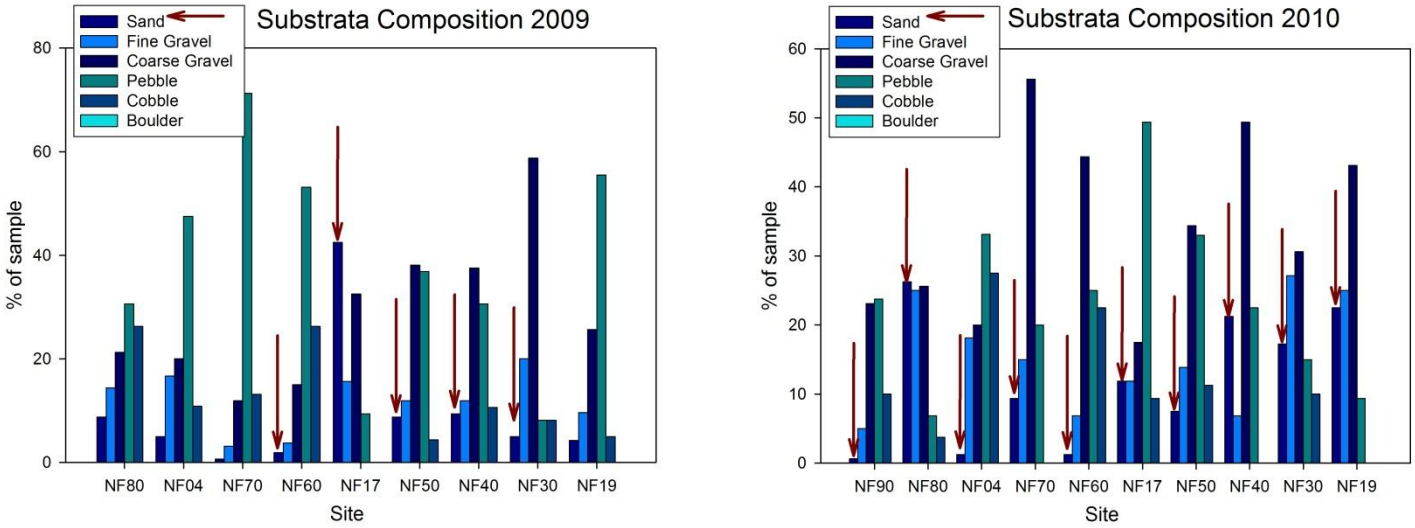


Figure 3.2. Substrata Composition. The average percent dominance of different substrate types sampled when sampling was stratified according to flow (Fig 3.1) is represented by bars. Sites are arranged from upstream to downstream. Both 2008 and 2009 are provided for reference.

[13] Please note that the scale of these measures is different from those used for geomorphology and habitat assessment. See "purpose statement" in the Executive Summary and Introduction sections of this document for further explanation.

[14] Particles in rivers are assorted by flow. In equilibrium, high flows scour smaller particles away and the dominant remaining particles are larger. In slower flows, the finer particles settle out and become more abundant as substrata. To estimate the site's actual substrate composition, a randomized transect regimen is required (a pebble count). This was not performed for this study; these values were determined by sampling a individual 1ft^2 benthic invertebrate samples, across a pre-selected (stratified sampling) range of flows.

[15] Where flow is the velocity of near-substrata water used to select samples from a consistent range of flows.

In 2009we observed a slight decrease in the amount of sand in benthic samples collected from NF30 and NF40. In 2010, the sand from samples at these sites was once again above the observed values from NF17 (East Fork River. ANOVA results indicated there was a significant difference in the amount of SAND[16] in samples from different sites in 2010 ($P<0.0001$). Tukey's LSD test indicated that the significant differences were numerous and differentiated the very low levels of NF90, NF04 and NF60, which had several zero PSI values because of interference by aquatic plants.

PARTICLE SIZE INDEX

The Particle Size Index (PSI) is a weighted average of the percent contribution of different substrate sizes (see Analytical Methods). It integrates all substrate types into an independent[17] measure which is known to correlate with macroinvertebrate community structure and abundance. Values of about 300-350 are ideal and are dominated by a complex mix of cobbles and other substrata. Values near zero are dominated by sand and silt. We found that in previous years, differences in community structure were strongly correlated with the PSI.

Although ANOVA results from 2009 indicated that NF17 had significantly smaller particle size than sites located below the (as well as above) the confluence of the east fork river, in 2010, there was no significant difference between the particle size of samples collected from the East Fork River or those collected below the confluence. However, the mean particle size did exhibit a trend of decreasing particle size downstream from the confluence (Fig. 3.3). Other sites stood out as having larger substrata on average; NF80, NF70 and NF60 were represented by samples with significantly higher PSI values than NF30 and NF40. This is an improvement from previous years where NF30 was lower and not significantly different from NF17.

This provides partial evidence for run-off mediated recovery of the New Fork River system from the accumulation of fines related to PAPA development combined with multiple years of below average flows.

[16] SAND is a variable used in analysis and was generated from field observations of the relative proportion of sand to other substrata in the top 10 cm of sample area. All capital letters are used to differentiate variable names from other nouns.

[17] Independence is an important consideration for statistics. Computational independence is especially important, since there is no way to hedge the null hypothesis to compensate. For example multi-colinearity problems would occur if we used all substrate classes for covariates; 100% cobble forces all other substrate size-classes to zero, and a zero percent cobble forces some other measures to non-zero values. Thus, the PSI not only helps explain change in invertebrate abundances, but also circumvents computational problems that would otherwise arise.

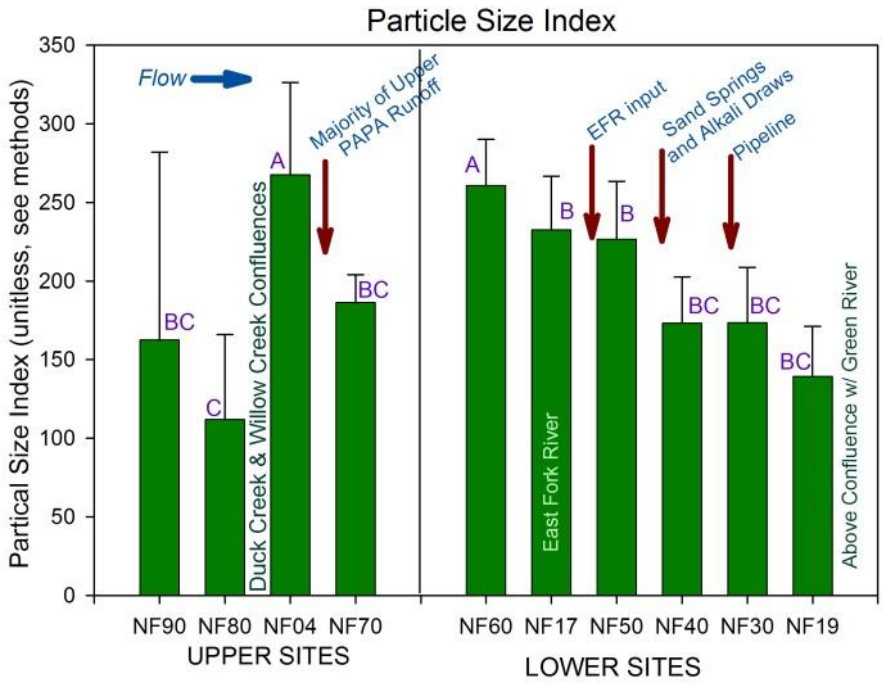


Figure 3.3. Particle Size Index. The particle size index indicated that benthic grabs (Surber samples) from NF17 had significantly lower mean particle size than samples collected from similar water velocity at all other sites (see text). Error bars are 95% confidence intervals. Letters are the final results of statistical tests (see Methods; Analytical Methods; Statistical Analyses, above); sites that share a letter are not significantly different from each other ($P<0.05$).

EMBEDDEDNESS

Embeddedness is the relative portion of course substrata buried among finer particles. It is a subjective measure expressed as the average percentage of larger particles buried among silt, sand, or very fine gravel. The impact of embeddedness on rivers and streams depends not only on the depth of embedding, but also the fineness of the embedding material and the extent of high embeddedness through the river-system. Finer material denies access to pore-spaces for invertebrates and small fish. If the material is organic in nature, it can deplete oxygen concentrations or facilitate plant growth.

One problem with the measure is that the nature of the embedding material is not quantifiable, and the measure is subjective. Different results are likely when two (or more) observers record observations for the same habitat—unless they have worked together extensively.

Embeddedness measures were too incomplete to use as analytical covariates in both 2009 and 2010 (Fig. 3.4); they are presented here only for general reference. We ran all statistical analyses with embeddedness as a covariate, and it was always excluded for lack of relevance to the biological data. Values were not available for several reasons (macrophyte beds, no large substrata to be embedded etc.). Since measures were reported in millimeters of fines around cobbles and pebbles, and since the standard "scrub" depth for Surber samples is 10 cm (100mm), samples consisting entirely of fine material could be reported as 100mm embeddedness. This will be discussed with the SCCD before changes are implemented. But if this is to be biologically relevant, it must be measured in a way where Embeddedness can account for instances where very fine substrata conceal larger particles, including macrophytes beds.

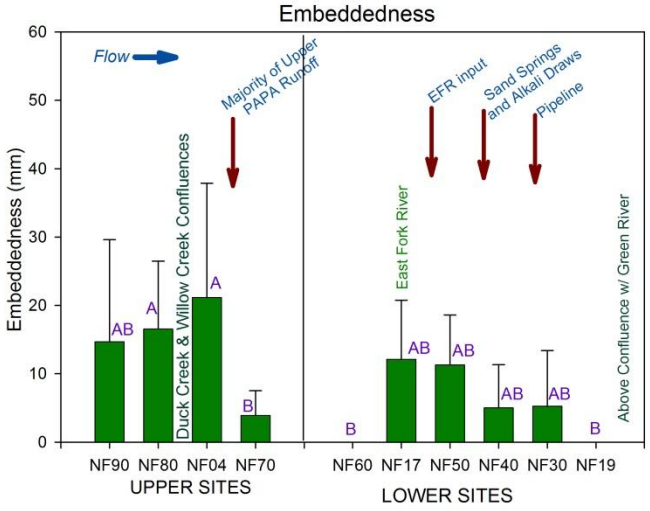


Figure 3.4. Embeddedness 2010. The average embeddedness of sampled (SS) substrate is represented by bars. Error bars represent 95% confidence intervals. Sites are arranged from upstream to downstream.

PLANT COVER

The proportion of plant cover was used as an explanatory variable for the first time in 2010. We found it was an especially useful covariate for several metrics. In cases where other covariates explained more variation among the metrics, and plant cover was not significant once those covariates were in the model, the metrics often left a similar impression as the figure below (Fig 3.5). Moreover, the statistical significance (as a grouping of similar sites) of metrics often closely aligned with this pattern, or with its reciprocal. This suggests that the role of plant material likely has a very strong influence on the distribution of macroinvertebrates in the New Fork River.

This measure should continue to be measured in the field and reported. Its explanatory use would be improved by expressing it as several variables: Vascular Hydrophyte cover, Moss Cover, Filamentous Algae Cover, and *Didymosphenia geminata* cover.

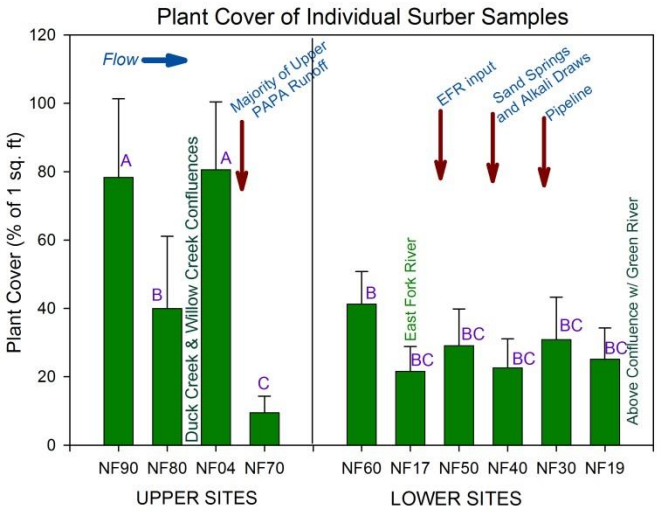


Figure 3.5. Plant Cover. The mean relative portion of plant cover from each square foot sampled for macroinvertebrates is presented with 95% confidence intervals. Sites that share a letter above their bars are not significantly different from each other (ANOVA, Tukey's HSD, P<0.05)

3.2 DIFFERENCES AMONG SITES (2010)

OVERALL DIFFERENCES AMONG SITES

The biological metrics were first screened for significant differences among sites using ANOVA (Table 3.1). Metrics which displayed a significant difference among sites were examined in greater detail to determine if the effects could be due to impairment related to development in the PAPA. All metrics except Taxa Richness and Non-insect abundance indicated that there was a statistically significant difference among the sites. ($P<0.05$, Table 3.1). Two columns are highlighted (NF40 and NF30) because they represent samples taken upstream and downstream from the pipeline area, and because previous years' results indicated that sediment-related changes occurred at these sites.

After running the GLM stepwise multiple regression, the means changed very little for most sites (Table 3.2). Usually, the sites that expressed very high or very low metric values changed the most where as more moderate metric values changed very little or not at all. This typically resulted in easier to interpret p-values and site groupings, but correcting for habitat variables did not ever change a hypothesized response to PAPA development to a non-response to PAPA development (or vise versa). The use of covariates helped explain the relative influence of natural variation among the sites and clarified the patterns identified by regular ANOVA.

TABLE 3.1. ANOVA Results. The ANOVA resulted in statistically significant differences among sites for all 13 metrics tested directly. Fishers LSD test was used to identify which sites were significantly different from each other. Sites that were significantly different from each other are noted by different letters in their columns. Sites that were not significantly different from each other share at least one letter with similar sites.

		Multiple Comparisons Grouping (Fisher's LSD)									
Metric	P-val	NF90	NF80	NF04	NF70	NF60	NF17	NF50	NF40	NF30	NF19
Taxa Richness	<0.001	AB	A	B	CD	CD	D	CD	C	CD	D
EPT- Richness	<0.001	A	AB	A	C	C	AD	CE	CE	CE	DE
% EPT	<0.001	A	AB	A	C	D	B	D	D	A	D
% Chironomid	<0.001	A	B	A	C	B	B	B	B	D	BD
% Non-Insect	<0.001	BE	C	BE	D	BDE	E	BD	BE	BE	BD
Dominance(5)	<0.001	A	AB	A	C	CD	B	CD	CD	CD	D
Gatherers	<0.001	A	A	A	E	CD	CB	D	B	B	CB
Filterers	0.007	BC	C	C	BC	C	AB	A	BC	C	BC
Collectors	<0.001	A	A	A	D	C	B	B	B	B	B
Scrapers	<0.001	D	D	D	A	B	C	C	C	C	B
Shredders	<0.00	C	C	C	A	B	B	B	B	B	B
Predators	<0.001	A	A	A	A	B	B	B	B	B	B
HBI	<0.001	AB	A	AB	E	C	D	CD	CD	BD	C
		Upper Study area				Lower Study Area					

TABLE 3.1. Habitat-Adjusted ANOVA Results. After correction for the variance explained by significant covariates, differences among the sites persisted. Although these data provided more explanation as to why communities were different, they did not result in statistical groupings that either obscured or reveled impacts to the New Fork River related to PAPA development. Fishers LSD test was used to identify which sites were significantly different from each other. Sites that were significantly different from each other are noted by different letters in their columns. Sites that were not significantly different from each other share at least one letter with similar sites.

		Multiple Comparisons Grouping (Fisher's LSD)									
Metric	P-val	NF90	NF80	NF04	NF70	NF60	NF17	NF50	NF40	NF30	NF19
Taxa Richness	<0.001	B	AB	B	A	A	AB	A	A	A	A
EPT Richness	<0.001	B	AB	B	A	A	A	A	A	A	A
% EPT	<0.001	B	B	B	A	A	A	B	BC	AC	AC
% Chironomid	<0.001	A	C	A	C	BC	BC	BC	**BC**	**B**	BC
% Non-Insect	<0.001	A	C	A	A	A	AC	A	A	AC	A
Dominance(5)	<0.001	A	A	A	B	B	A	AB	**AB**	**AB**	AB
Gatherers	<0.001	A	A	A	B	B	AB	BC	AB	AC	AB
Filterers	<0.001	AB	AB	AB	AB	AB	AB	B	AB	A	AB
Collectors	<0.001	A	A	A	B	BC	A	A	A	A	C
Scrapers	<0.001	B	B	B	A	AB	B	B	B	B	B
Shredders	<0.00	C	C	C	A	B	BC	BC	BC	BC	BC
Predators	<0.001	A	A	A	A	B	B	B	B	B	B
HBI	<0.001	A	A	A	B	BC	BC	BC	BC	B	BC
		Upper Study Area				Lower Study Area					

TAXA RICHNESS

The samples contained averages between 15-18 species (taxa) for each site. This is slightly fewer than in 2008 because the data were truncated to operational taxonomic units (OUTs) to prevent richness inflation by ambiguous taxonomic identities. The results are similar to those observed in 2009, when we used the same OTUs (Table 3.1, Fig. 3.5).

Differences among the observed taxa richness values for the upper study area reflected the greater richness at the downstream site, NF70, which was statistically significantly greater than all other upper study area sites. The other upper study area sites were not significantly different from each other. This response does not indicate any impairment related to development in the PAPA.

Differences among the lower study area were more subtle and only differentiated the site with the greatest richness (NF40) from the two sites with the lowest richness (NF17, NF19). All the other sites were not significantly different from each other. Since much of the content of previous reports has focused on the changes potentially related to PAPA development at NF30, it is worth pointing out that taxa richness at NF30 was not significantly different from the other sites in the lower study area.

Analysis of covariance indicated that the metric was significantly correlated with FLOW (P=0.026) and moderately correlated with a FLOW*PSI interaction term (P=0.069). The GLM analysis was re-run to account for this variation and the differences among sites remained highly significant (P<0.001) and the model's adjusted means were very close to the non-adjusted means . Moreover, accounting for the influence of flow on taxa richness did not change the groupings of statistically similar/different sites.

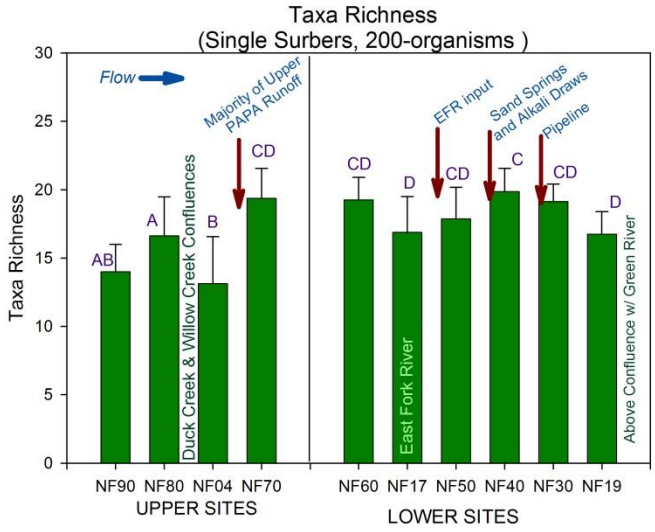


Figure 3.6. Taxa Richness. There were no statistically significant changes in taxa richness of the upper or lower study areas that would indicate impairment related to human activities in the PAPA. Sites sharing a common letter were not significantly different from each other.

EPT RICHNESS

In the upper study area, EPT richness varied between 8-13 taxa on average and the only significant differences occurred between NF70 and the other sites of the area, all of which were not significantly different from each other.

The lower study area sites exhibited a similar range of EPT richness values as the upper study area and the only statistically significant differences among sites were between the site with the greatest richness (NF60) and the two sites with the lowest richness(NF17, NF19). The other sites were not significantly different from each other. Since much of the content of previous reports has focused on the changes potentially related to PAPA development at NF30, it is worth pointing out that taxa richness at NF30 was not significantly different from the other sites in the lower study area.

Analysis of covariance indicated the metric was significantly correlated with FLOW (P=0.012) and moderately correlated with a FLOW*PSI interaction effect (P=0.07). The GLM analysis was re-run to account for this variation and the differences among sites remained highly statistically significant afterwards (P<0.001) and the models adjusted means were very similar to the non-adjusted means. The adjustment was generally less than 0.5 taxa. The sites of the lower study area all had their average EPT richness increased slightly. The only significant changes involved NF19 and NF17. NF19 was no longer significantly different from NF90 and NF70 (both of the upper study area). NF17 was no longer significantly different from the other lower study area sites, except NF60. Thus, although accounting for the influence of flow did not change the findings of the initial statistical tests, it did re-enforce the similarity of downstream sites in the richness of EPT orders.

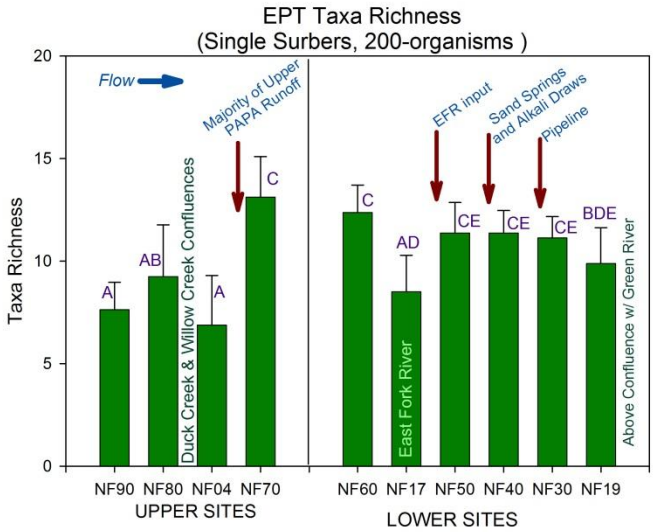


Figure 3.7. EPT Richness. There were no statistically significant changes in EPT taxa richness of the upper or lower study areas that would indicate impairment related to human activities in the PAPA. Sites sharing a common letter were not significantly different from each other.

EPT ABUNDANCE

The EPT orders comprised nearly 60% of all the organisms at NF70, whereas all the other sites had EPT taxa representing between about 15%-45% of all the organisms in the samples. High values of EPT relative abundance are desirable because many of these insects are more sensitive to pollution and sedimentation than many other groups of invertebrates.

The upper study area had significant difference in EPT relative abundance between NF70 and all other sites, the other sites were not significantly different from each other. This pattern of differnces in EPT relative abundance does not indicate any impairment related to PAPA development.

The lower study area found significant differences between NF17 (East Fork River) and the other sites, and between NF30 and the other sites. The sites NF60, NF50, NF40 and NF19 were not significantly different from each other. We expected NF17 to have lower EPT relative abundance because of the predominance of sandy substrata. The site NF30 had lower EPT relative abundance than all the sites of the lower study area, including NF17. The lower relative abundance of EPT insects was offset by greater relative abundances of midges and worms (as shown by the metrics % Chironomidae and % non-insects below). This finding reiterates the findings of earlier reports from the years 2006-2008 (but not 2009), where a significant change in community structure occurred between NF40 and NF30. During all these years the change involved a decrease in the relative abundance of sensitive organisms and an increase in sediment tolerant organisms. Although the difference in EPT abundance among lower study sites was statistically significant, NF30 was not significantly different from the upper study area sites NF90, NF80, and NF04. This helps to keep the magnitude of the difference in perspective.

Analysis of covariance indicated the metric was significantly correlated with FLOW (P=0.008) and with a FLOW*PLANTS interaction effect (P=0.004). The GLM analysis was re-run to account for this variation and the differences among sites remained highly statistically significant afterwards (P<0.001) and the models adjusted means were very similar to the non-adjusted means. The adjusted means increased the upper study area sites NF90, NF80, and NF04, while lowering NF70 slightly (NF70 remained significantly different from all upper-study area sites). The lower study area was more homologous after controlling for the influence of flow and its interaction with aquatic plants. NF60 and NF19 were not significantly different from each other but both supported significantly greater EPT relative abundance compared to NF17 and NF30; none of the other lower sites were significantly different from the others.

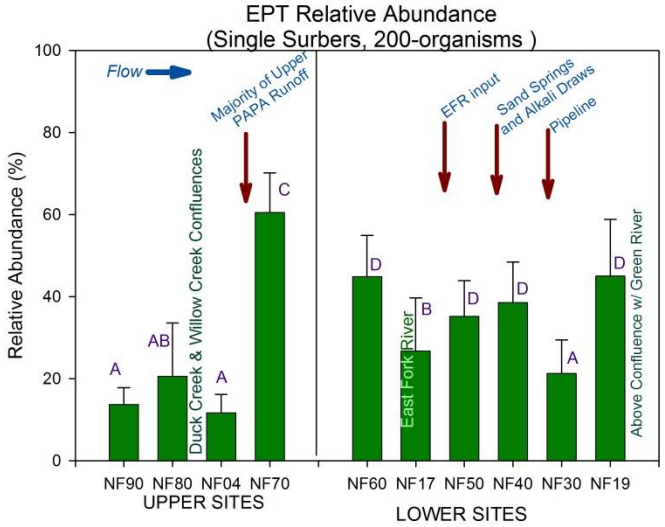


Figure 3.8. EPT Relative Abundance. There were no statistically significant changes in EPT relative abundance of the upper or lower study areas that would indicate impairment related to human activities in the PAPA. Sites sharing a common letter were not significantly different from each other.

CHIRONOMIDAE ABUNDANCE

The non-biting midges of the family Chironomidae are represented by about 4000 species in North America, with at least 40 species known from the New Fork River. Midges have a wide range of habitat references, tolerances and ecological requirements; however, when they increase in dominance it is usually considered an indicator of ecological perturbation.

The upper study area had a wide range of chironomid relative abundance from about 10-60% of the communities' samples on average. This spans range of normal values for larger rivers with fine sediments and vascular plant beds. NF90 and NF04 were not significantly different from each other, but both were significantly greater than NF80 and NF70. Both NF70 and NF80 were significantly different from each other. The very low relative abundance observed at NF70 was due to the very high relative abundance of EPT orders. Because NF04 is not significantly different from NF90 (our upstream reference and WY DEQ regional reference site) these findings do not reflect ecological perturbation related to development on the PAPA.

Analysis of covariance indicated the metric was significantly correlated with PLANTS (P=0.025). Differences among sites remained highly significant (P<0.001) even after correcting for the variation related to plant cover among sites (Fig. 3.9). The upper study area produced a lower (-5%) adjusted mean for NF80 and increased adjusted mean at NF70 (+5%), with the net effect that NF90 and NF04 were both significantly greater than NF80 and NF70, which were not significantly different from each other. The effects were less strongly pronounced in the lower study area, and the analysis indicated that there were no statistically significant differences among the lower study areas.

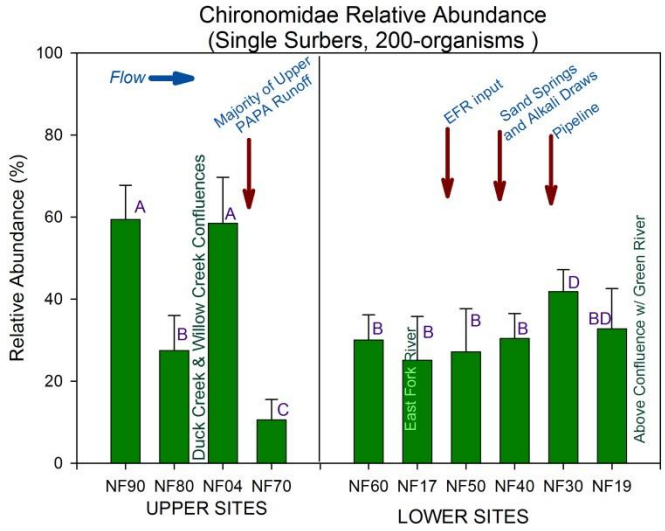


Figure 3.9. Chironomidae Relative Abundance. There were no statistically significant changes in relative abundance of chironomid midges from the upper or lower study areas that would indicate impairment related to human activities in the PAPA. Sites sharing a common letter were not significantly different from each other.

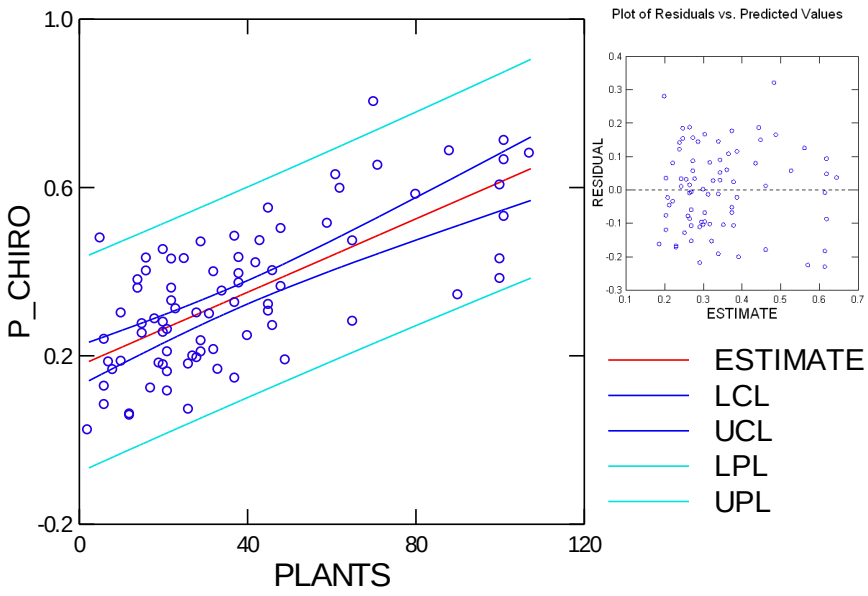


Figure 3.10. Chironomidae Abundance and Plant Cover. There was a relatively strong relationship between the abundance and density of chironomid midges and the portion of plant cover recorded in the field ($P<0.001$; $r^2=0.42$). The analysis passed tests of normality (Kolgorov-Smirnov test $P> 0.05$) , homogeneity of variances (Levene's Test $P>0.05$), and residuals (inset) did not suggest problems with multi-colinearity or non-linearity.

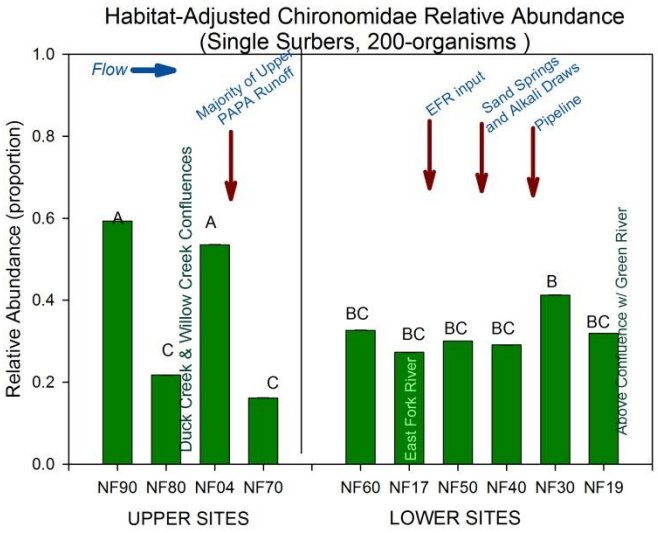


Figure 3.11. Chironomidae Relative Abundance. The covariance means adjusted for the influence of plant cover were very similar to non-adjusted means (Fig. 3.9). This is the desired outcome; very large changes would indicate poor study design unless one was more interested in differences related to plants than differences due to location. The differences among sites were simpler; there were no significant differences among the sites comprising the lower study area. The upper study area had high midge abundance at the upper reference which was not significantly different from NF04 and another reference had much lower midge-abundance but was not significantly different from NF70. Note that generally we expect midge abundance to remain below 60% and lower scores are considered "better."

NON-INSECT ABUNDANCE

Aquatic insects are dominant invertebrates in terms of abundance, diversity, biomass and production of typical North American freshwater ecosystems. When non-insects dominate invertebrate assemblages, it indicates something somewhat unusual, often disturbance. The dominance of non-insects in the New Fork River has been relatively high since about 2004. As the monitoring program developed to encompass increasing development in the PAPA, we identified one particular area of concern at NF30. This site is below a pipeline crossing and several drilling pads had encroached into the riparian area upstream from this site. In 2008 and 2007 we found non-insect abundances were abnormally high and highly correlated with fine sediments, which appeared to be elevated in high velocity reaches (indicating active erosion processes). In 2009, the first year with an above average spring runoff, the abundances of non-insects were reduced much lower numbers.

In 2010, we had a spring runoff that was about equivalent to the 52-year average in both magnitude and duration. We also observed a moderate increase in non-insect abundance at some sites. The upper study area had the site with the greatest abundance of non-insects, mostly *Nais* sp. worms, at site NF80. It also had the site with the lowest average abundance of non-insects, NF70. The other upper sites, NF90 and NF04 were not significantly different from each other nor from any of the lower study area sites.

At the lower study area, samples collected from NF17 (East Fork River) had the greatest average abundances of non-insects (which was significantly less than NF80 from the upper study area), but it was significantly different from sites NF50 and NF19. None of the other sites were significantly different from each other.

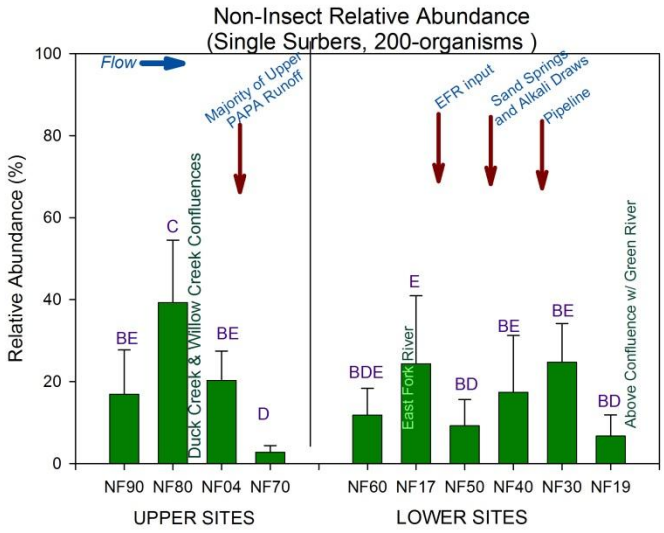


Figure 3.12. Non-Insect Abundance. There was a variety of differences among the sites in non-insect abundance during the 2010 field sampling season. However, none of the differences observed from the upper or lower study areas indicate impairment related to human activities in the PAPA. Sites sharing a common letter were not significantly different from each other

Other than the unexplained increase in non-insects at NF80, this response profile does not suggest impairment related to development at the PAPA. This site had an average relative abundance of non-insect taxa more than twice the levels observed previously. We recommend a site reconnaissance to determine if these findings are related failure of erosion controls or riparian encroachment. The invasive nuisance algae, *Didymosphenia geminata*, has been found at some sites in the study area and thick coverings of the algae have been linked to declines of aquatic insect abundance and increases in midge and non-insect abundance of the Kootenai River in Montana (Marshall 2006). Although the Kootenai River is different from the New Fork in many ways, this does establish that it can influence the abundance of some indicator organisms, such as midges and non-insects.

Analysis of covariance indicated the metric was significantly correlated with some spurious interaction effects. Once these were removed from the model, the only significant variable was the relative amount of Plant cover (P=0.025). The effects of this covariate were most influential in the upper study area, presumably because plants were more abundant there. The means adjusted for the influence of plant cover were decreased by nearly 10% at NF90, and NF04, whereas NF70's non-insect abundance was adjusted upwards about 5% after correction for plant-cover. NF80 did not change significantly by adjusting for amount of variance related to plant cover. The lower study area sites had their relative abundance of non-insects increased between about 0-2%. The adjusted model resulted in NF80 having significantly greater abundance of non-insects than all sites other than NF19 and NF30. There were no other significant differences among the upper and lower study areas after adjusting for aquatic plant-cover.

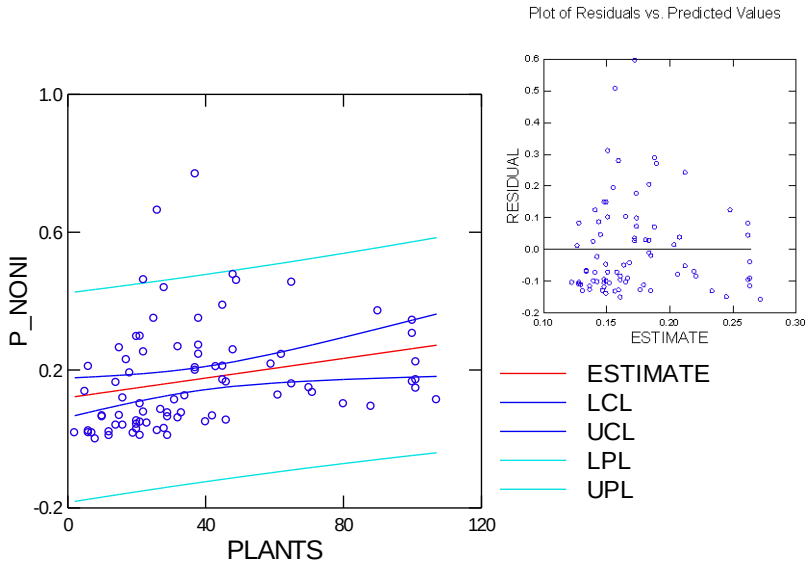


Figure 3.13. Non-Insect Abundance and Plant-Cover. There was a significant relationship between the abundance and density of non-insects and the portion of plant-cover recorded in the field (P<0.021; r2=0.021). However, this relationship was less influential than the influence of PLANTS on midge abundance (Fig. 3.9). The analysis passed tests of normality (Kolgorov-Smirnov test P> 0.05) , and homogeneity of variances (Levene's Test P>0.05). However, the residuals (inset) indicated there could be slight non-linearity issues.

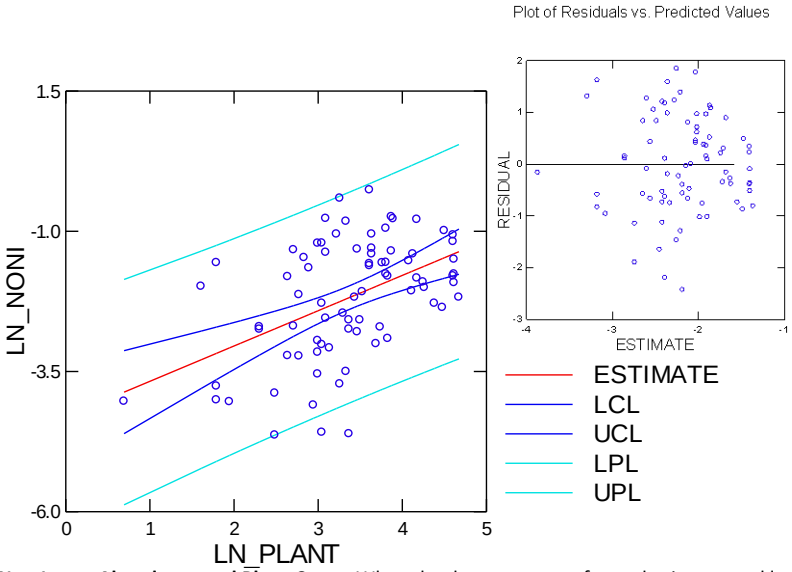


Figure 3.14. Non-Insect Abundance and Plant-Cover. When the data were transformed using natural logarithms, the fit improved (P<0.001, r2=0.225) However, this relationship was less influential than the influence of PLANTS on midge abundance (Fig. 3.9). The analysis passed tests of normality (Kolgorov-Smirnov test P> 0.05) , and homogeneity of variances (Levene's Test P>0.05). The residuals (inset) indicated that non-linearity issues were addressed though this transformation.

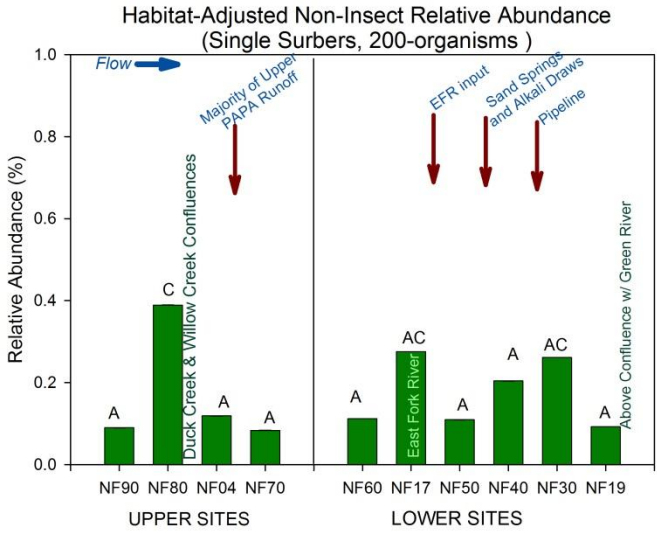


Figure 3.15. Non-Insect Abundance. Both the raw plant-cover covariate and the transformed plant-cover covariate produced the same statistical groupings with only very small differences among adjusted means (<1%). However, none of the differences observed from the upper or lower study areas indicate impairment related to human activities in the PAPA. Sites sharing a common letter were not significantly different from each other

HILSENHOFF BIOTIC INDEX (HBI):

The HBI rates streams by the abundance-weighted average organic-pollution-tolerance of the entire species assemblage. Thus, a score of 10 occurs only when the entire invertebrate assemblage is extremely tolerant of organic enrichment[18], whereas a score of zero indicates that the entire community is composed of only very sensitive organisms. Generally streams of the Wyoming Basin should have values less than about 4.0[19]. The HBI values obtained in 2010 were higher than in 2009 for all sites and the upper study area (NF90, NF80, NF04) had average scores near 6, which is higher than desired. The high dominance of midges at these sites was probably very influential because the collective OTUs assigns them an HBI tolerance of 6.0. As with 2009, the best HBI value was observed from NF70.

The HBI metric reflects other differences in water quality that are apparently unrelated to development in the PAPA. For example, the high values at the two farthest upstream sites (NF80 and NF04) are likely responding to the high-conductivity tributaries a short distance upstream from NF80, or responding to the high density of plants (which occur in productive waters).

The HBI did not indicate a significant impairment related to PAPA development.

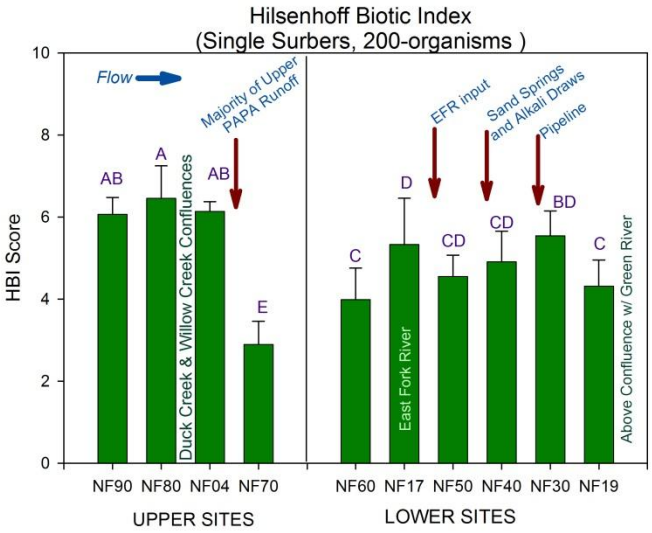


Figure 3.16. Hilsenhoff Biotic Index (HBI). High values indicate communities dominated by pollution-tolerant organisms. Bars are averages of eight samples, and 95% confidence intervals. Sites sharing letters above bars are not significantly different from each other.

[18] In reality this would only occur in a pile of sewage sludge or similar enriched, anaerobic environment.

[19] Hargett and ZumBerge (2006) report 95% of reference streams had HBI values greater than 2.7, our earlier studies (Marshall 2006, 2007, 2008, 2009) found that New Fork River sites often have HBI values ~4.0.

Analysis of covariance indicated the metric was significantly correlated with FLOW (P=0.031) and a FLOW*PLANTS (P=0.018) interaction effect. The difference among sites remained highly significant (P<0.001). The effects of these covariates were most influential in the upper study area, presumably because plants were more abundant there. The means adjusted for the influence of plant cover were decreased.

After correction for significant covariates, there were no significant differences among the sites comprising the lower study area. The upper study area had one site (NF70) that had significantly lower HBI scores (indicating better water quality) than the other upper sites.

None of these findings suggest impairment of the New Fork River biota in relation to development in the PAPA.

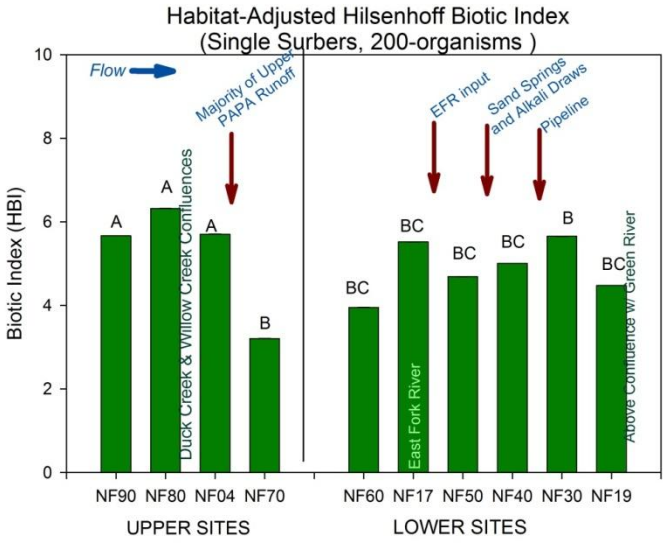


Figure 3.17. Adjusted Hilsenhoff Biotic Index (HBI). High values indicate communities dominated by pollution-tolerant organisms. Bars are averages of eight samples, and 95% confidence intervals. Sites sharing letters above bars are not significantly different from each other.

DOMINANCE (5 TAXA)

Communities are generally considered healthier if they support a diverse assemblage of species. In diverse ecosystems there are many species, whereas non-diverse systems have high relative abundance of just a few species. Thus the five most-abundant species comprise a greater proportion of the community in low-diversity locations than at high-diversity communities. Stribling et al. (2000) identified the dominance of five taxa should be less than about 80-85% for streams in the Wyoming Basin Ecoregion.

In the upper study area, NF90 (91.6%) and NF04 (91.4%) had >90% dominance. Site NF80 averaged 86% dominance. NF70 was closer to the expected level of dominance with the five most-abundant taxa comprising 72.3% of the community. There were no differences among NF90, NF80, NF04, but NF70 was had significantly lower dominance than the other upper study area sites.

The lower study area had a more homologous level of community dominance than the upper study area. Although the sites generally had greater dominance than observed in 2009, the 2010 dominance values were within the expected range defined by Stribling et al. (2000). Although the differences among sites were small, so was the variation within sites. This resulted in small differences becoming statistically significant. East Fork River (NF17) samples were not significantly different from NF30 but both these sites had significantly greater dominance than the other lower sites which were not significantly different from each other.

Analysis of covariance indicated the metric was significantly correlated with the relative amount of plant cover (P=0.042), with site differences remaining statistically significant (P<0.001). The effects of this covariate were most influential in the upper study area, presumably because plants were more abundant there. Plant cover increased the dominance of the five most abundant taxa and after adjusting for this influence, the upper site means which exceeded 90% were moderated and NF70 had its mean dominance increased slightly by the covariance model. However, the differences among upper sites remained unchanged after adjustment. After adjustment for the plant cover, no significant differences persisted among the lower study area sites.

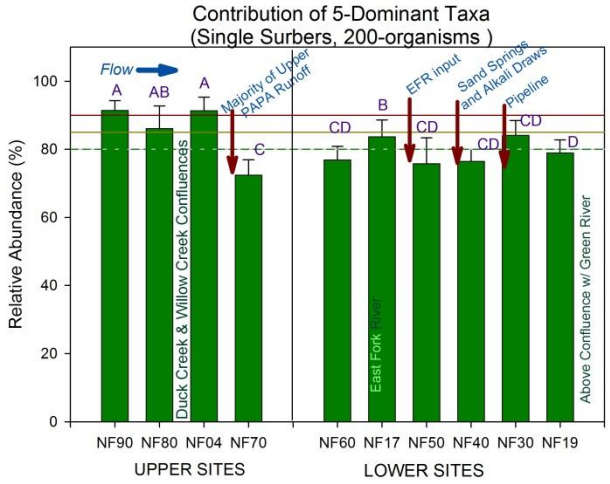


Figure 3.18. Dominance(5). The combined relative abundance of the five-most-abundant taxa (Dominance(5)) was used a measure of community dominance. The only significant difference among sites was NF70, which was significantly lower dominance (good) than all other sites. Error bars are 95% confidence intervals.

3.3 ABUNDANCE OF FUNCTIONAL FEEDING GROUPS (2010)

Modern aquatic ecology is often concerned with the movement of carbon (energy) through food webs. Thus it is useful to group organisms by their roles in processing organic material; their so-called "functional feeding group." Anthropogenic disturbances that alter the function of food webs, are intended to be prevented under the auspices of the Clean Water Act and are sometimes manifested by shifts in the abundance of organisms of different functional feeding groups.

Collector-gatherers are organisms that search out deposits of fine particulate organic material for sustenance. Collector-filterers remove fine particulate organic material from the water column for the same purpose. In streams and rivers, the amount of suspended organic material is linked to the velocity of water. Since both collector-gatherers and collector-filterers consume fine particles of organic matter, the differences in their relative abundances may be related to localized flow conditions. For this reason, it is often useful to consider the combined abundance of filterers and gatherers as the "collector" group[20].

The relative abundance of Collectors was greatest at highly vegetated sites of the upper study area were they comprised about 90% of the invertebrates. The three farthest upstream sites had significantly more collectors than NF70. The Lower Study area had the upstream reference (NF60) exhibiting significantly fewer collectors than all other sites that were not significantly different from each other. No covariates explained a significant portion of the variation in the abundance of collecors.

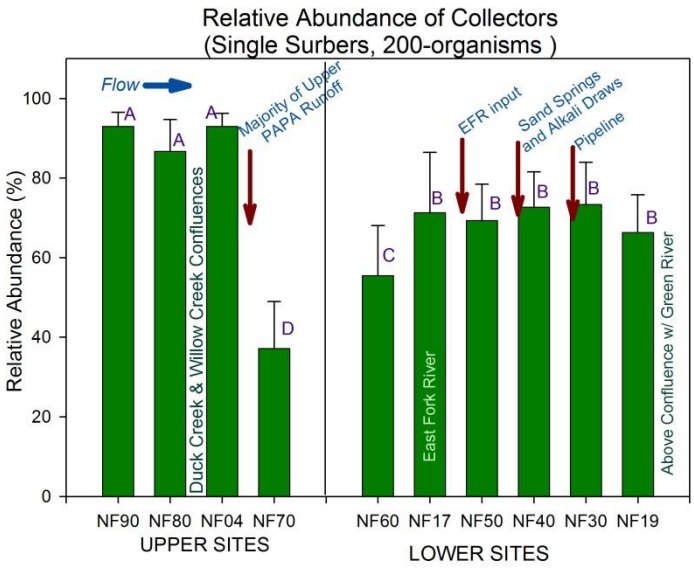


Figure 3.19. Collectors. The combined relative abundance collector-gatherers and collector-filterers. There did not appear to be any significant difference related to PAPA development. The upper study area had significantly fewer collectors at NF70. In the lower study area, NF60, was significantly less-dominated by collectors than the other sites. Error bars are 95% confidence intervals.

[20] I refer to this as "combined collectors" to avoid confusion with either of the other collector functional feeding groups (either gatherers or filterers).

3.4 CHANGES OVER TIME (2007-2010)

Many sites now have amassed four years of data using improved sampling methods. It becomes more useful to assess trends over time. This eventually will become the primary method of identifying impairments related to activities in the PAPA. This is essential given the limitations of the WSII to assess streams in Sublette County for change.

The following line graphs show how the sites have changed over time for select metrics. The metrics based on relative abundance were selected because these are not likely to be significantly affected by the implementation of conservative operational taxonomic units in 2009 and 2010. For this reason, taxa richness, and EPT richness were not examined in this way until the historic database is completely developed.

Thus the metrics EPT abundance, percent non-insect, percent Chironomidae, percent collectors, percent scrapers, percent dominance (5), and HBI were used to assess changing community structure over time. Changes in other functional feeding groups are presented in Appendix 2 (supplementary graphs), but the major trends occurring over this time period can be surmised by examination of the metrics introducing this paragraph.

The figures show for each site the mean metric value with standard errors. For the final summary, the change from 2007 to 2010 for each metric at each site was assigned a score of -1, 0, or +1, depending if the change was consistent with declining, no-change, or improving water quality, respectively. Then the net sum of the scores for each metric was reported to describe the net change as positive or negative.

EPT RELATIVE ABUNDANCE

The relative abundance of EPT taxa is expected to decline as water quality declines and as more tolerant organisms become more dominant. Thus, when this metric declines, it implies a decline in water quality[21], whereas an increase in the relative abundance of EPT taxa can occur when (or where) water quality improves. The relative abundances of EPT organisms are naturally variable, but values near or above 50% of the community are not uncommon. Values <20% suggest there is a smaller than normal portion of these sensitive organisms in the community for some reason.

All sites had a lower portion of the community represented by EPT insect orders in 2010 (Fig 3.20). For all the sites, except for NF80, the change from 2009 to 2010 was a statistically significant decrease in EPT relative abundance, suggesting declines in New Fork River's condition. However, 2010 was not always significantly different from 2007: NF70, NF17, NF50, and NF40 were not different from 2007. Those that were significantly different from 2007 in 2010 (NF04, NF60, NF30, and NF19) exhibited a significant decline relative to the initial survey.

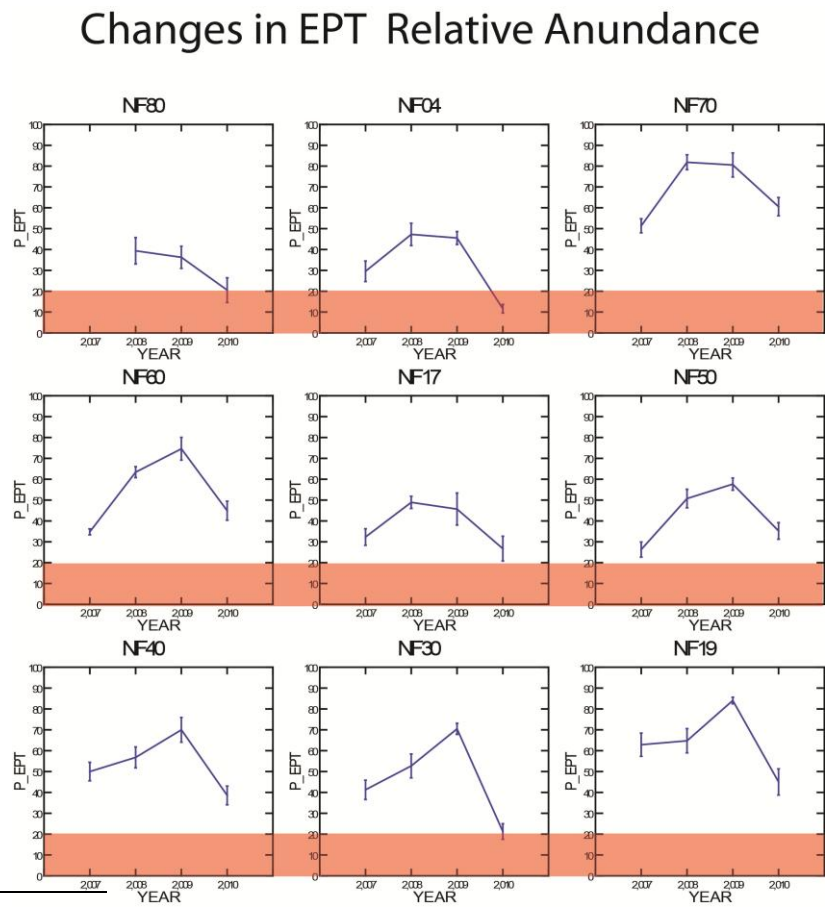


Figure 3.20. EPT Abundance over Time.
The relative abundance of EPT orders declined in 2010. Error bars are 1 SEM.

[21] In this context, water quality includes physical conditions of the stream as well as chemical conditions.

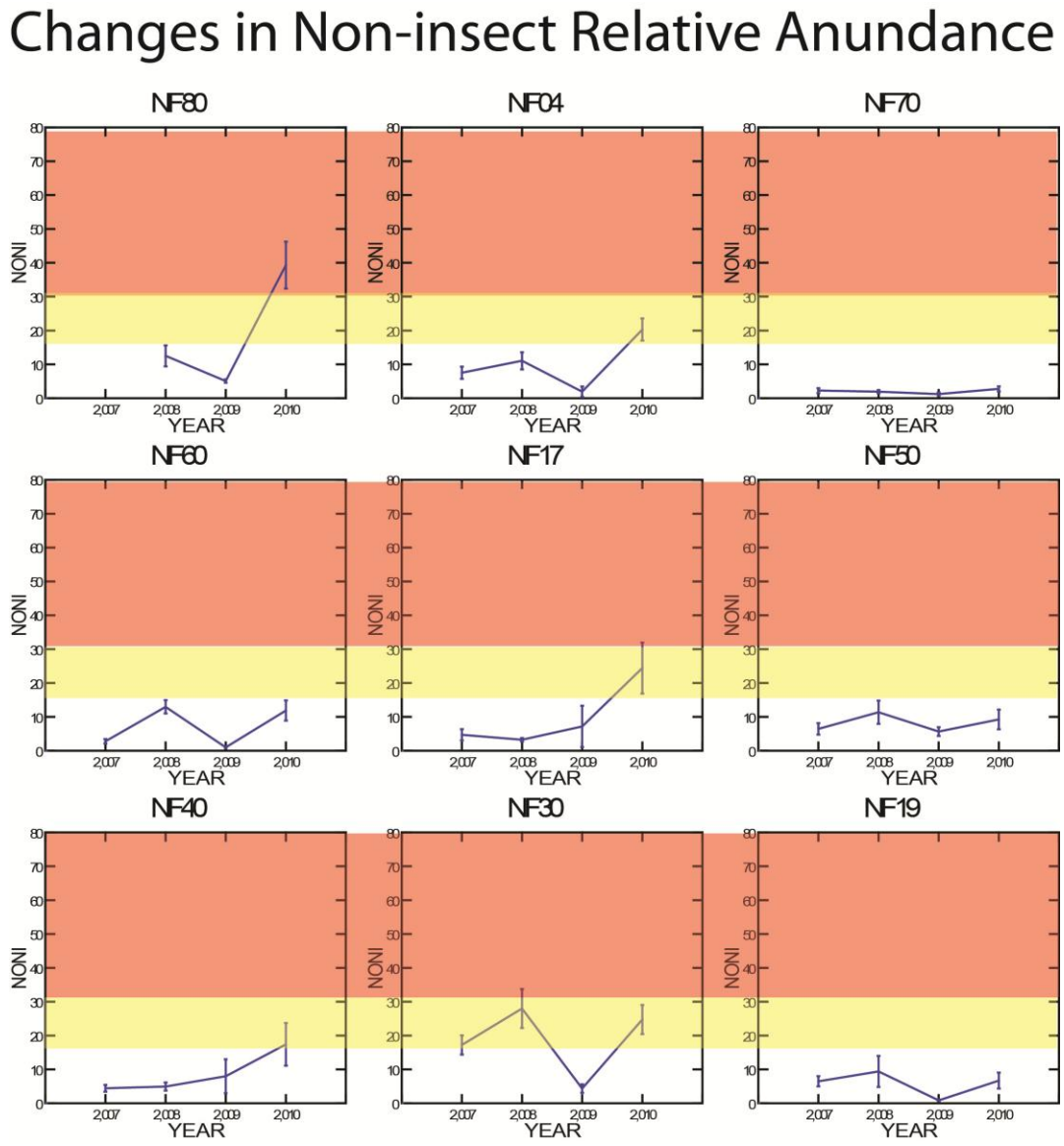


Figure 3.21. Change in Non-Insect Abundance (percent). The abundance of EPT taxa generally increased in 2010. The figures show average values ±1 Standard Error of the mean (SEM) 2007-2010. The SEM is about ½ the confidence interval.

CHANGE IN THE ABUNDANCE OF NON-INSECTS

Most North American freshwater rivers are dominated by aquatic insects--both in terms of abundance and diversity. Communities that are dominated by non-insect macroinvertebrates occur in unusual circumstances, and may indicate ecological perturbation or naturally saline environments. Thus increases in the %Non-insect metric are considered undesirable, under most circumstances and may indicate declines in water quality. Since we have noted a problem with this metric over recent years, we were especially hopeful that the 2010 survey would result in lower densities of non-insects in the New Fork River.

Considering only changes from 2009 to 2010, five of the nine sites (NF80, NF04, NF60, NF17, NF30) showed a significant increase in non-insect abundance in 2010 (Fig. 3.21); most of these sites exhibited significant declines in 2010 (relative to 2009). Changes in non-insect abundance at sites NF70, NF50, NF40, and NF19 were not statistically significant. The site with the greatest contribution of non-insects ~40% (NF80) also showed the most change (almost entirely *Nais*).

A study I conducted (Marshall 2006) on the Kootenai River in Montana found that worms and midges dominated benthic communities where the nuisance algae *D. geminata* becomes prevalent (>8mg/cm). We have found evidence of this species of algae at some locations in the New Fork River.

CHANGE IN THE ABUNDANCE OF CHIRONOMID MIDGES

Non-biting midges (Chironomidae) are a very diverse group of macroinvertebrates, with over 4,000 species in North America. Although some species are very sensitive to disturbance and/or pollution, generally midges become dominant in streams that are disturbed or polluted. Thus significant increases in midge abundance can indicate a decline in water quality. The shaded threshold around 60% midges is based on my experience with fine sediment rivers.

Over the three years sampled prior to this report, midges generally declined over time at most sites. In 2010, most sites exhibited increased abundance of midges in the community. Six of the nine sites (NF04, NF60, NF50, NF40, NF30, NF19) exhibited statistically significant increases in midge abundance from 2009 to 2010 (Fig. 3.22). The changes at the other three sites from 2009 to 2010 were not statistically significant.

The role of D. geminata needs to be accounted for in future surveys to ensure that PAPA development is not erroneously blamed for changes associated with the spread of this species.

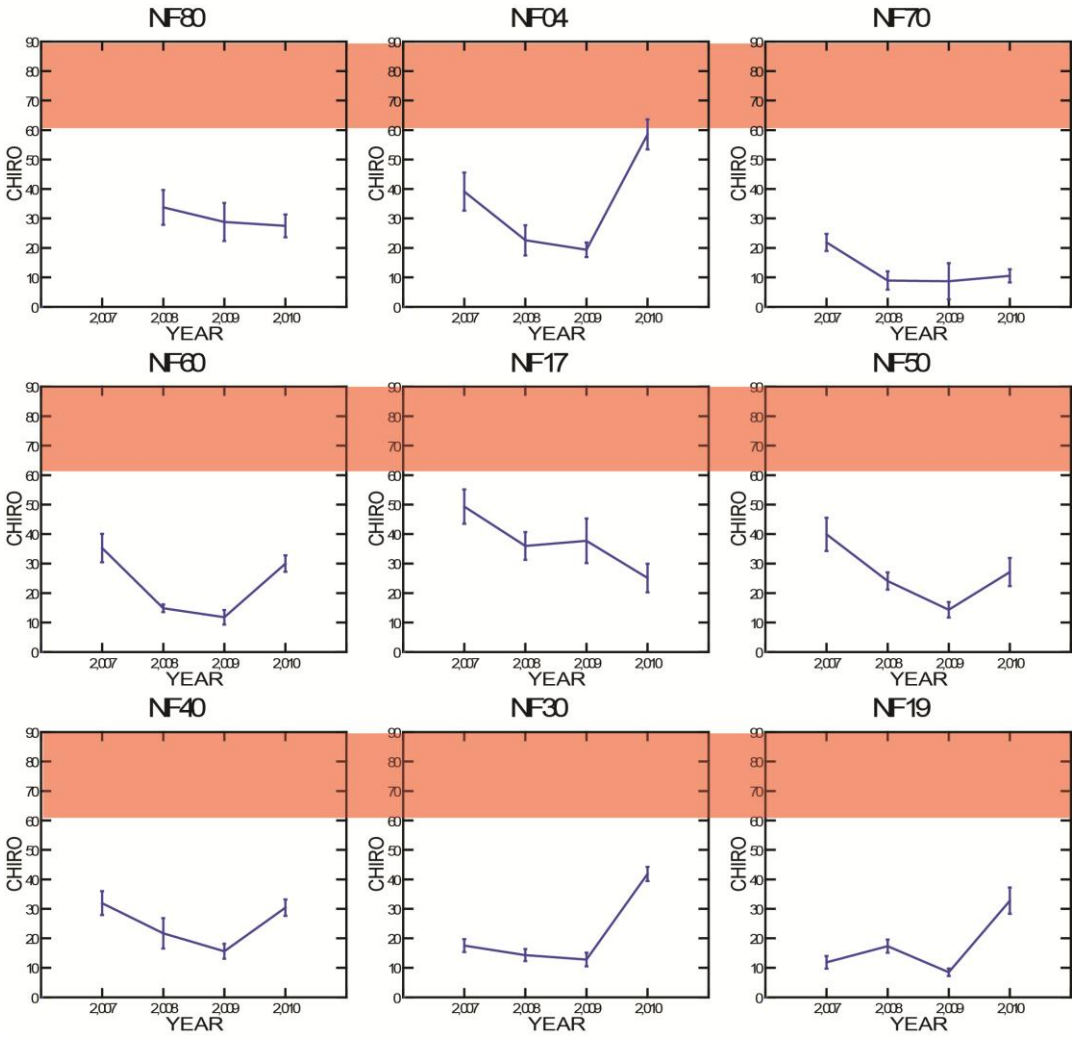


Figure 3.22. Change in Chironomidae Abundance (percent). The abundance of chironomid midges generally increased in 2010. The only site to exhibit an abundance of midges greater than 50% was NF04. Of the sites with significant increases in 2010 (NF04, NF60, NF50, NF40, NF30, NF19), most were preceded by significant decreases in 2009. Many sites were not significantly different from 2007's midge abundance at the site. We're most concerned about the midge abundance at NF04, NF30 and NF19, but D. geminata may be influencing these communities. The figures show the SEM, and means of four years 2007-2010.

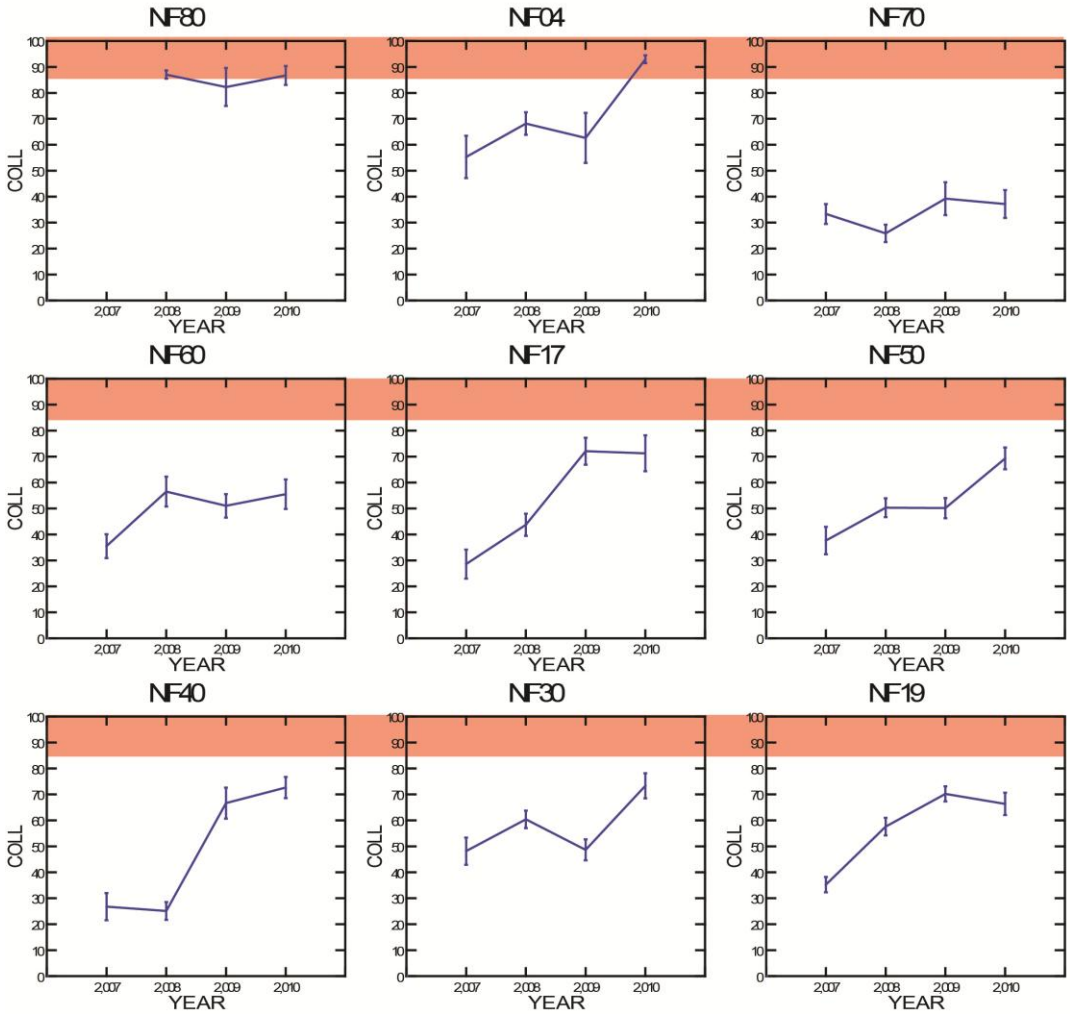


Figure 3.23. Change in Collector Abundance (percent). The abundance of collectors showed a general trend of increasing collector abundance. Values approaching 85-90% collectors indicate that all the other functional groups together comprise less than 10%. In transitional streams like the New Fork River, we expect scrapers shredders and predators combined to comprise 20-40% of the community. The loss of these groups could indicate a serious impairment of ecological dysfunction. The figures show the standard errors (SEM), and means of three years 2007, 2008, 2009 and 2010.

CHANGES IN COLLECTOR ABUNDANCE

The percent contribution of collectors should respond similar to midges. That is, increases in water quality should result in a decrease in collector abundance, whereas declines in water quality can cause an increase in the abundance of collectors and more specialist functional groups (such as shredders and scrapers) decline.

Only three of the sites (NF04, NF50, NF30) changed significantly since 2009; each with a significant increase in collector abundance. Of these sites, we are most concerned with the extremely high abundance of collectors at NF04, where the average contribution of collectors exceeded 90% (this is possibly caused by the presence of *D. geminata* at NF04).

Although NF19 did not increase significantly in 2010, the value did increase incrementally each year before 2010. We expected this response as suspected sediment impacts on the stream would be become mediated by downstream sediment export, with a temporary moderate increase in collectors, midges, and non-insects. This could eventually occur at the farthest downstream site (NF19) either ephemerally as the upstream. All the sites below the East Fork River (NF17) have gradually increased their abundance of collectors over time to about 70%.

CHANGES IN DOMINANCE (5)

Healthy ecosystems usually support more diverse ecosystems than disturbed ecosystems (with some exceptions). Declines in water quality often allow a small group of invertebrates to dominate the ecology of the system; resulting in an increase in dominance. Thus, when monitoring for change, increases in dominance is usually considered to be indicative of declines in the condition and function of the resource. The dominance of the 5 most abundant taxa has become a standard in many areas because it is believed to be somewhat less variable than measures including fewer taxa, and more responsive than measures using more taxa.

Over the four years of replicated data, most sites showed either insignificant change or a slight increase (Fig 3.24). NF04 was the only site to have a significant increase that pushed the community dominance above the >85-90% Wyoming Basin impairment threshold defined by Stribling et al. (2000). This metric was marginally increased by use of optimized taxonomic units, which provided a conservative estimate of richness-resulting in fewer taxa of slightly more abundance[22].

[22] This is a computational concern that only affects the results if they are compared with other studies using different methods. For example, if some OTU's truncate a group of species to the genus level to prevent metric inflation, the result would be that a group of 3 species and 1 genus would be reduced to one larger taxon, 1 genus, with an abundance value comprised by the sum of the three species (and the genus). This effects richness measures and can to a lesser extent also influence measures of dominance. Comparisons should use the same OTU's (Appendix 1).

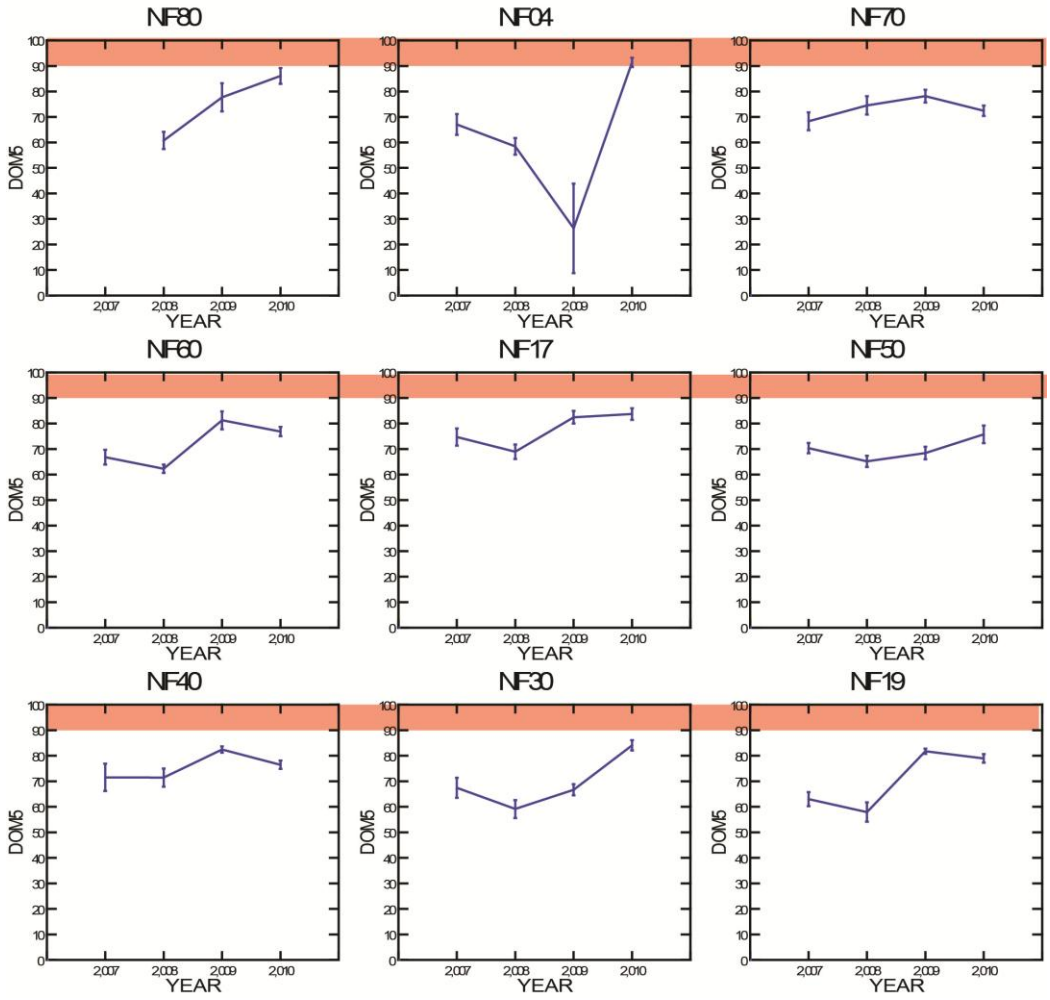


Figure 3.24. Contribution of Dominant (5) Taxa. The percent contribution of the 5 dominant taxa changed only slightly in 2010 at most sites. However NF04 exhibited a very large increase and exceeded the expected range for non-impaired streams (Stribling et al. 2000). Error bars are 1 SEM.

CHANGES IN THE HILSONHOFF BIOTIC INDEX

Declines in HBI values indicate that the community has become increasingly represented by species that are known to be sensitive to organic pollution. Conversely, increases occur when communities are dominated by species that are more tolerant to pollution. In many cases, organic pollution was also accompanied by sedimentation when species tolerance values were generated. We have found that the metric is responsive to both sediment and organic enrichment.

In 2010, every site showed a statistically significant increase in HBI; an indication of significant decline in the taxonomic composition of macroinvertebrates of the New Fork (and East Fork) River (Fig 3.25). However, in most cases, the HBI values observed in 2010 were not significantly greater than those observed in 2007. The two exceptions were NF80 and NF04, which were sufficiently dominated by tolerant organisms elevating the index above 6.0 because most organisms were tolerant to organic enrichment.

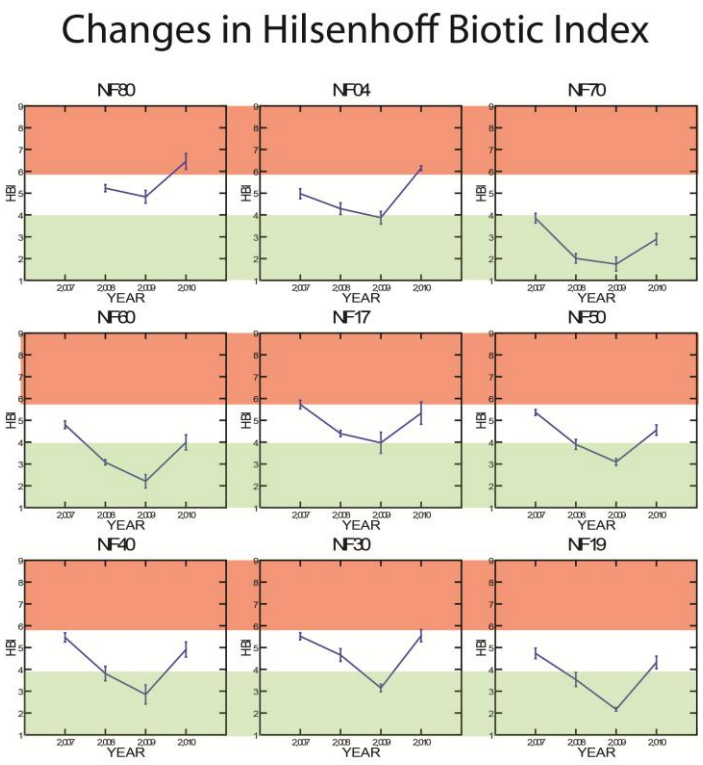


Figure 3.25. Hilsenhoff Biotic Index (HBI).
The HBI changed significantly at all sites in 2010, but it was generally not significantly greater than the values observed in 2007. Exceptions include NF04 and NF80. Error bars are 1 SEM.

CHANGES IN SCRAPER ABUNDANCE

Scrapers are specialists that scrape algae from large substrata as sustenance. The Wyoming DEQ's Wyoming Stream Invertebrate Index sets the regional goal for the relative abundance of scrapers at >33-34% (Stribling et al. 2000, Hargett and Zumburge 1996) with the mathematical assumption that more is better[23]. Over the four year study period, most sites showed an increase in the abundance of scrapers (Fig. 3.26).

Scrapers were most abundant at NF70, where they were mostly represented by glossosomatid caddisflies (*Glossosoma* sp. and *Protoptila* sp., with *Glossosoma* clearly dominant). These are clean, obligate scrapers and probably not indicative of any form of pollution. NF70 was the only site in which we observed communities with significantly more scrapers in 2010; all other sites were not significantly different from 2007 (essentially baseline for this sampling regimen).

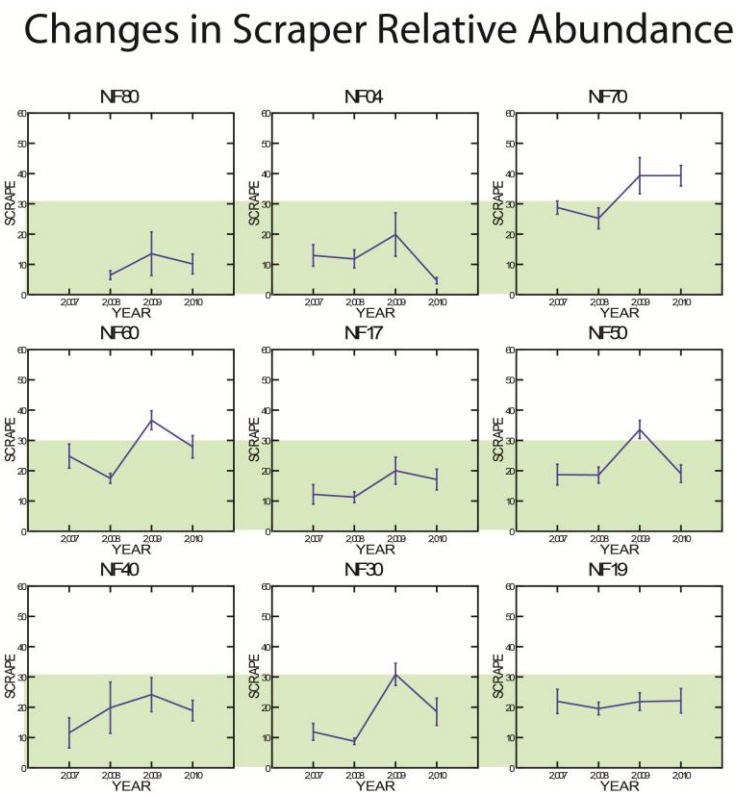


Figure 3.26. Scraper Abundance.
Relative abundance of scrapers declined significantly at three sites (NF04, NF50, and NF30). The value at NF04 is most relevant because it was caused the extreme dominance of collectors (Fig. 3.23). Other sites did not change significantly in 2010. Error bars are 1 SEM.

[23] However, in cases of nutrient enrichment the scrapers become dominant as a sign of ecological perturbation. There are also issues of multicolinearity when using both scraper abundance and collector abundance (100% scrapers = 0% collectors) as a measure of assessment. Scrapers are presented for descriptive and diagnostic purposes. Indeed, a community of 40-50% scrapers would be highly unusual and most likely a sign of very high primary production.

3.5 CHANGES FROM 2009-2010

The general trend among all the sites in the New Fork River exhibited biological metrics that suggested declining conditions from 2009 to 2010 (Table 3.2). In 2009, all sites except the East Fork River (NF17) exhibited a net improvement in environmental conditions. However, in 2010, all sites exhibited a net decline in condition.

The site NF04, stands out as being significantly impaired in all 7 of the temporal metrics we examined (Table 3.2). Moreover the community was >90% dominated by collectors, the contribution of the 5 most-abundant taxa exceeded 90%, and the HBI score was >6.0. The benthic of community appears to be responding to disturbance(s). NF04 receives flow from Duck and Willow Creeks as well as from upstream and adjacent lands.

3.6 OVERVIEW OF CHANGES FROM 2007-2010

Analysis of change since 2007 produced several metrics at some sites which suggested improved water quality and the net effect of these indicated that NF50, NF60, and NF70 improved more than they declined (Table 3.3). However, NF04 showed declines in water quality in all seven temporal measures. This indicates that NF04 was under more ecological stress in 2010 than any time since the sampling program began.

TABLE 3.2. Summary of Change from 2009 to 2010. This table cross-tabulates trends from the previously discussed metrics in terms of the hypothesized response to changes in water quality. Measures that suggest improving water or declining water quality were noted with the symbols ☺ or ☹ respectively. Metrics indicating no change over time were noted with the symbol 😐. For example, the relative abundance of EPT orders (%EPT) is expected to *increase* with improving water quality so sites that showed a significant increase in EPT are noted with the symbol ☺. The Hilsenhoff Biotic Index *decreases* with improving water quality, which is indicated with the ☺ symbol; had any of the HBI values increased over time, they would have been noted by the ☹.

SITE	% EPT	% Non-Insect	% Midges	% Collectors	% Scrapers	% Dominance (5)	HBI	
NF80	😐	☹	😐	😐	😐	😐	☹	**-2**
NF04	☹	☹	☹	☹	☹	☹	☹	**-7**
NF70	☹	😐	😐	😐	😐	😐	☹	**-2**
NF60	☹	☹	☹	😐	😐	😐	☹	**-4**
NF17	☹	☹	😐	😐	😐	😐	☹	**-3**
NF50	☹	😐	☹	☹	☹	😐	☹	**-5**
NF40	☹	😐	☹	😐	😐	😐	☹	**-3**
NF30	☹	☹	☹	☹	☹	😐	☹	**-6**
NF19	☹	😐	☹	😐	😐	😐	☹	**-3**
	-8	-5	-6	-3	-3	-1	-9	

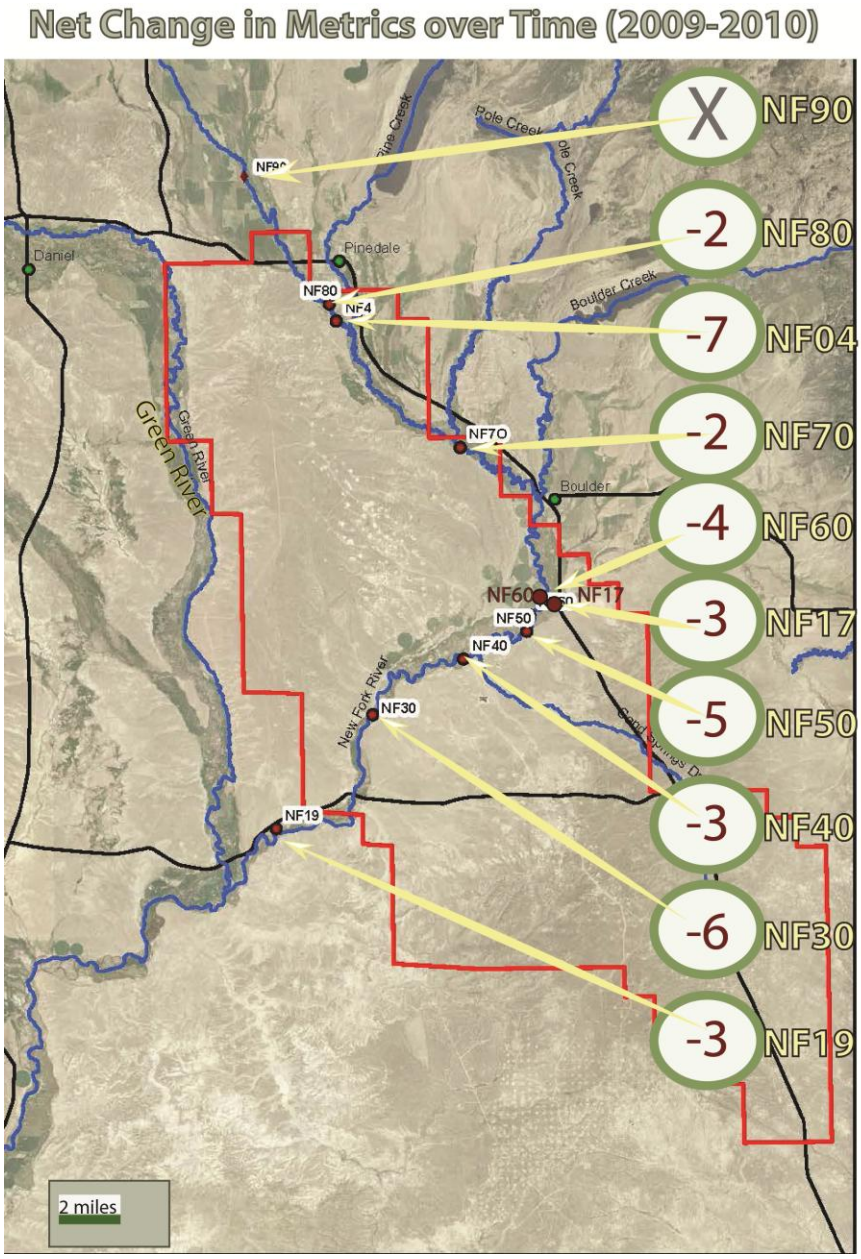


Figure 3.27. Schematic of Sites and Net Changes among Sites.
This resulted in a net change score. Negative numbers indicate that more metrics indicated a decline in conditions in 2010. All site showed a net decline in benthic community structure. The changes were most severe at NF04 and at NF30 (near sites of riparian encroachment).

TABLE 3.3. Summary of Change from 2007 to 2010. This table cross-tabulates trends from the previously discussed metrics in terms of the hypothesized response to changes in water quality. Measures that suggest improving water or declining water quality were noted with the symbols ☺ or ☹ respectively. Metrics indicating no change over time were noted with the symbol 😐. For example, the relative abundance of EPT orders (%EPT) is expected to *increase* with improving water quality so sites that showed a significant increase in EPT are noted with the symbol ☺. The Hilsenhoff Biotic Index *decreases* with improving water quality, which is indicated with the ☺ symbol; had any of the HBI values increased over time, they would have been noted by the ☹.

SITE	% EPT	% Non-Insect	% Midges	% Collectors	% Scrapers	% Dominance (5)	HBI	
NF80	☹	☹	😐	😐	😐	☹	☹	**-4**
NF04	☹	☹	☹	☹	☹	☹	☹	**-7**
NF70	😐	😐	☺	😐	☹	😐	☺	**+1**
NF60	☺	😐	😐	😐	😐	😐	☺	**+2**
NF17	😐	☹	☺	☹	😐	☹	😐	**-2**
NF50	😐	😐	☺	☹	😐	😐	☺	**+1**
NF40	😐	☹	😐	☹	😐	😐	😐	**-2**
NF30	☹	😐	☹	☹	😐	☹	😐	**-4**
NF19	☹	😐	☹	☹	😐	☹	😐	**-4**
	-3	-4	0	-6	-2	-5	+1	

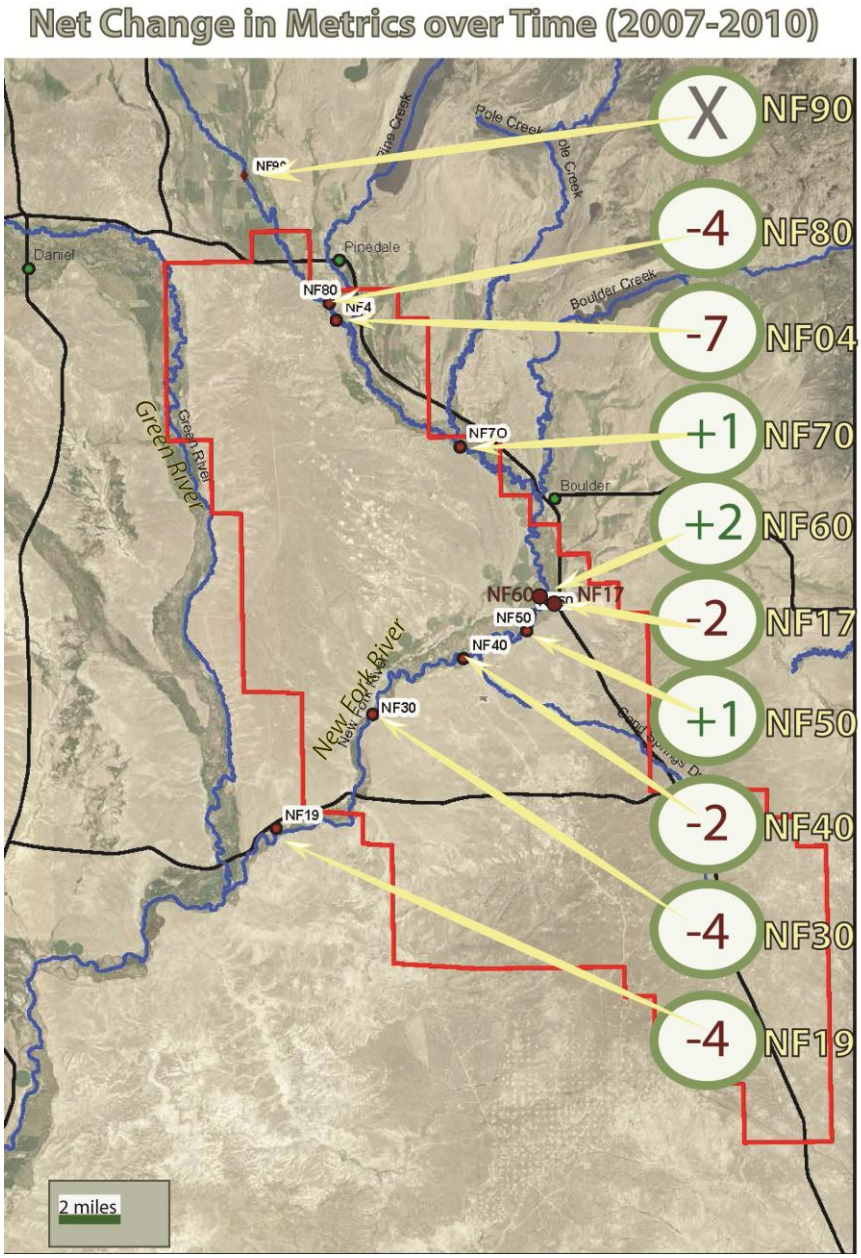


Figure 3.27. Net Change relative to 2007. This figure shows the change relative to 2007 (the first year with all sites replicated). Sites with more statistically significant increases in quality than declines are positive numbers. Negative numbers indicate the site's condition has significantly declined since the 2007 field season. Note that negative changes in all seven metrics occurred at NF04.

4.0 DISCUSSION

4.1 RESULTS SUMMARY (2010)

The field survey described by this report collected eight 1-sq. ft. samples from each of nine sites on the New Fork River and one reference on the East Fork River, (NF17). The invertebrates of these samples were identified in the laboratory and used to calculate metrics that are used to evaluate the ecosystem function.

Analysis of spatial trends did not identify any particular longitudinal shifts in community structure that could be associated with development in the PAPA. There was a significant correlation on midges and non-insects (mostly *Nais*) with a new field covariate that summarized the relative plant cover of each square foot sample. Particle size was not as explanatory as plant cover or near-substrata flow.

We also compared 2010 to findings from earlier years to assess change over time. Analysis of changes since 2009 indicated that every site showed a decline in the ecological condition based on the net change in 7 ecological summary measures. One site, NF04 declined significantly in all 7 measures and had several measures with values beyond ecological impairment thresholds. The causes of these changes were once again related to dominance of non-insects, and midges—both of which were strongly correlated with plant cover.

Analysis of change since 2007 produced several metrics at some sites which suggested improved water quality and the net effect of these indicated that NF50, NF60, and NF70 improved more than they declined. However, NF04 showed declines in water quality in all seven temporal measures. This indicates that NF04 was under more ecological stress in 2010 than any time in the since the sampling program began.

All the metrics indicating ecological design at NF04 were related to increases in midges and worms. For instance, the increase in collectors and dominance was caused by the increased relative abundance of midges and worms. Similarly, since collectors comprised over 90% of the community at NF04, scrapers were forced to comprise less than 10%.

It is important to note that these changes appear to be correlated to plant cover in 2010, and unless there is some reason that PAPA development could increase plant growth; these changes are most likely caused by some influence other than natural gas development in the PAPA. Most likely, It is related to spread and growth of mats of the nuisance alga *Didymosphenia geminata*. The changes were correlated with plant cover, and mats of *D. geminata* were not differentiated from vascular hydrophytes in the field data we received from the 2010 sampling season. However, we did observe some clumps of the species in preserved macroinvertebrate samples in the laboratory, so we have evidence of its prevalence. We even found midges that have cells of the species in their digestive tracts (a note of record I believe).

In an unrelated study (Marshall 2007) on the Kootenai River in Montana, I found a very similar response pattern and was able to quantify the relationship between the mats the alga forms and the functional feeding groups negatively affected by the dominance of midges and worms which burrow in the mucilaginous slime formed among the mats of cells (Fig 4.1).

4.2 RECOMMENDATIONS (2010)

To ensure that PAPA developers are not unjustly blamed for the effects of these algae, the program should begin to quantify the abundance of epileptic biofilm mass so that it can be accounted for as a covariate in our analyses.

This should be done by collecting the mucilage from the substrata of a known area, and quantifying it in the laboratory. The area could be defined with a piece of 1" PVC pipe and a washer. We already have the lab equipment for these analyses and could do conduct the analysis for a small budget ($25 per algae sample, with 3 algae samples representing each Surber sample recommended). Data would be dry weight per cm^2.

Since samples from 2011 have just been collected, the sites could be re-visited with 10-12 representative samples collected from areas similar to those from which the samples were collected at each site and overnight shipped on ice. Again, to document that these impairments at NF04 are not related to PAPA development, it is important to document the actual level of change related to *D. geminata*, which should be expected to spread to other sites in the New Fork River.

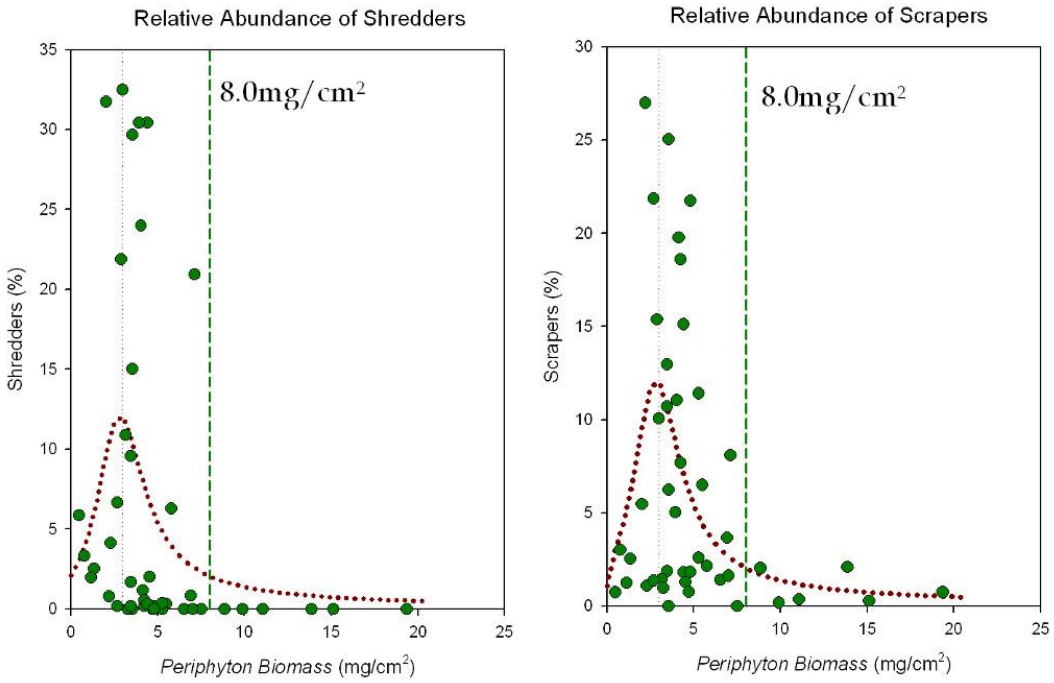


Figure 4.1. Effects of D. geminata on Functional Feeding Groups of the Kootenai River, Montana. Both shredders and scrapers esentially disapeard from locations where the density of the alga exceeded 8mg/cm^2. The patern in the abundance of worms and midges along with their correlation with plant cover (including D. geminata) suggest that the impairment at NF04 may be due to a similar community-level response.

5.0 LITERATURE CITED

Hargett, E.G. and J, R. ZumBerge. 2006. Redevelopment of the Wyoming Stream Integrity Index (WSII) for Assessing the Biological Condition of Wadable Streams in Wyoming. Wyoming Department of Environmental Quality, Cheyenne WY.

Hart D. D. and C. M. Finelli. 1999. Physical-biological coupling in streams: The pervasive effects of flow on benthic organisms. Annual Review of Ecology and Systematics. 30: 363-395.

Fonseca, D. M. and D.D. Hart. 1996. Density-dependent dispersal of black fly neonates is mediated by flow. OIKOS 75(1):49-58.

Marshall, B.D. 2005a. An Assessment of the Biological Condition of the New Fork River, near the Pinedale Anticline Project Area, Sublette County, Wyoming: 2004 Survey Results. Prepared for Sublette County Conservation District

Marshall, B.D. 2005b. An Assessment of Chemical and Biological Trends in the New Fork River Basin, Sublette County, WY: December 2005 Survey Results. Prepared for Sublette County Conservation District.

Marshall, B.D. 2006. An Assessment of the Biological Condition of the New Fork River, near the Pinedale Anticline Project Area, Sublette County, Wyoming: 2005 Survey Results. Prepared for Sublette County Conservation District and the Pinedale Anticline Water Task Group, Pinedale Wyoming.

Marshall, B.D. 2007. Effects of the nussance alage on benthic community structure of the Kootenai River, below Libby Dam, Montana. Prepared for Montana Department of Fish Wildlife and Parks, Libby Montana.

Marshall, B.D. 2007. An Assessment of the Biological Condition of the New Fork River, near the Pinedale Anticline Project Area, Sublette County, Wyoming: 2006 Survey Results. Prepared for Sublette County Conservation District and the Pinedale Anticline Water Task Group, Pinedale Wyoming.

Marshall, B.D. 2008. An Assessment of the Biological Condition of the New Fork River, near the Pinedale Anticline Project Area: 2007. Prepared for Sublette County Conservation District and the Pinedale Anticline Water Task Group, Pinedale Wyoming.

Marshall, B.D. 2009. Assessment of the Biological Condition of the New Fork River, in the Vicinity of the Pinedale Anticline Project Area: 2008. Prepared for Sublette County Conservation District, Pinedale, WY.

McCune, B., and M. J. Mefford. 1995. Multivariate analysis on the PC-ORD system. Version 2.0. MjM Software, Gleneden Beach, Oregon, USA.

Stribling, J.B., B. K. Jessup. J. Gerritsen. 2000. Development of biological and physical habitat criteria for Wyoming streams and their use in the TMDL process. Prepared for U.S. Environmental Protection Agency, Region VIII, Denver Co.

ter Braak, C. J. F. 1986. Canonical correspondence analysis: a new eigenvector technique for multivariate direct gradient analysis. Ecology 67:1167-1179.

ter Braak. C. J. F. 1994. Canonical community ordination. Part I: Basic theory and linear methods. Ecoscience 1:127-140.

WYDEQ. 2008b. WYOMING'S METHOD FOR DETERMINING WATER QUALITY CONDITION OF SURFACE WATERS AND TMDL PRIORITIZATION CRITERIA FOR 303(d) LISTED WATERS. Wyoming Department of Environmental Quality, Water Quality Division, Watershed Section. http://deq.state.wy.us/wqd/watershed/Downloads/305b/Final%20Methodology.pdf

Zar, J. H. 1999. Biostatisitcal Analysis (4th edition). Prentice Hall Pub. Upper Saddle River, NJ 07458.

ZumBerge, J. 2004. Personal contact. Wyoming Department of Environmental Quality, Sheridan, WY. An MS Excel file of bioassessment criteria, modifying the WSII for all ecoregions.

Hargett, E.G., J.R. ZumBerge and C.P. Hawkins.2005. Development of a RIVPACS Model for Wadeable streams of Wyoming. Wyoming Deptartment of Environmental Quality, Cheyenne, WY.

Appendix 1. Operational taxonomic units for 2009.

Note that as the database is built these may change.

ORDER	FAMILY	Taxon	O.T.U.
EPHEM	**Baetidae**	**Baetidae**	Baetidae
EPHEM	**Baetidae**	*Acentrella sp.*	Acentrella
EPHEM	**Baetidae**	*Acentrella insignificans*	Acentrella
EPHEM	**Baetidae**	*Acentrella turbida*	Acentrella
EPHEM	**Baetidae**	Acerpenna pygmaea	Acerpenna
EPHEM	**Baetidae**	Acerpenna sp.	Acerpenna
EPHEM	**Ameletidae**	Ameletus sp.	Ameletus
EPHEM	**Leptohyphidae**	Asioplax sp.	Asioplax
EPHEM	**Ephemerellidae**	Attenella margarita	Attenella
EPHEM	**Ephemerellidae**	Attenella sp.	Attenella
EPHEM	**Baetidae**	Baetis bicaudatus	Baetis
EPHEM	**Baetidae**	Baetis flavistriga	Baetis
EPHEM	**Baetidae**	Baetis sp.	Baetis
EPHEM	**Baetidae**	Baetis tricaudatus	Baetis

EPHEM	**Caenidae**	Caenis youngi	Caenis
EPHEM	**Baetidae**	Callibaetis sp.	Callibaetis
EPHEM	**Baetidae**	Centroptilum sp.	Centroptilum
EPHEM	**Heptageniidae**	Cinygmula sp.	Cinygmula
EPHEM	**Baetidae**	Diphetor hageni	Diphetor
EPHEM	**Ephemerellidae**	Drunella coloradensis/flavilinea	Drunella
EPHEM	**Ephemerellidae**	Drunella doddsi	D. doddsi
EPHEM	**Ephemerellidae**	Drunella grandis	Drunella
EPHEM	**Ephemerellidae**	Drunella spinifera	Drunella
EPHEM	**Heptageniidae**	Epeorus albertae	Epeorus
EPHEM	**Heptageniidae**	Epeorus deceptivus	Epeorus
EPHEM	**Heptageniidae**	Epeorus grandis	Epeorus
EPHEM	**Heptageniidae**	Epeorus longimanus	Epeorus
EPHEM	**Heptageniidae**	Epeorus sp.	Epeorus
EPHEM	**Ephemeridae**	Ephemera sp.	Ephemera
EPHEM	**Ephemerellidae**	**Ephemerllidae**	Ephemerellidae
EPHEM	**Ephemerellidae**	Ephemerella inermis/infrequens	Ephemerella

EPHEM	**Ephemerellidae**	Ephemerella sp.	Ephemerella
EPHEM	**Heptageniidae**	Heptagenia sp.	Heptagenia
EPHEM	**Heptageniidae**	Heptageniidae	Heptageniidae
EPHEM	**Baetidae**	Heterocloeon sp.	Heterocloeon
EPHEM	**Leptophlebiidae**	Leptophlebiidae	Leptophebiidae
EPHEM	**Leptophlebiidae**	Leptophlebia sp.	Leptophebia
EPHEM	**Heptageniidae**	Nixe sp.	Nixe
EPHEM	**Leptophlebiidae**	Paraleptophlebia sp.	Paraleptophlebia
EPHEM	**Baetidae**	Plauditus sp.	Pladitus
EPHEM	**Heptageniidae**	Rhithrogena sp.	Rithogena
EPHEM	**Ephemerellidae**	Serratella tibialis	Serratella
EPHEM	**Leptohyphidae**	Tricorythodes sp.	Tricorythodes
ODONATA	**Coenagrionidae**	Coenagrionidae	Coenagrionidae
ODONATA	**Gomphidae**	Ophiogomphus sp.	Gomphidae
ODONATA	**Ophiogomphus**	Gomphidae	Gomphidae
PLECOP	**CAPNIIDAE**	Capniidae	Capniidae
PLECOP	**Chloroperlidae**	Chloroperlidae	Chloroperlidae
PLECOP	**Perlidae**	Claassenia sabulosa	Claassenia

PLECOP	**Perlodidae**	Cultus sp.	Cultus
PLECOP	**Perlidae**	Hesperoperla pacifica	Hesperoperla
PLECOP	**Perlodidae**	Isoperla sp.	Isoperla
PLECOP	**Nemouridae**	Malenka sp.	Malenka
PLECOP	**Perlodidae**	Perlodidae	Perlodidae
PLECOP	**Pteronarcyidae**	Pteronarcella sp.	Pteronarcella
PLECOP	**Pteronarcyidae**	Pteronarcys californica	Pteronarcys
PLECOP	**Pteronarcyidae**	Pteronarcys sp.	pteronarcys
PLECOP	**Perlodidae**	Skwala sp.	Skwala
PLECOP	**Perlodidae**	Sweltsa sp.	Sweltsa
PLECOP	**Taeniopterygidae**	Taeniopterygidae	Taeniopterygidae
PLECOP	**Nemouridae**	Zapada cinctipes	Zapada
PLECOP	**Nemouridae**	Zapada columbiana	Zapada
PLECOP	**Nemouridae**	Zapada oregonensis gr.	Zapada
HEMIP	**Corixidae**	Sigara sp.	Sigara
COLEO	**Dytiscidae**	Agabus sp.	Agabus
COLEO	**Dytiscidae**	Brychius sp.	Brychius
COLEO	**Elmidae**	Cleptelmis addenda	Cleptelmis

COLEO	**Hydrophilidae**	Enochrus sp.	Enochrus sp.
COLEO	**Hydrophilidae**	Helophorus sp.	Helophorus sp.
COLEO	**Hydrophilidae**	Laccobius sp.	Laccobius sp.
COLEO	**Hydraenidae**	Ochthebius sp.	Ochthebius sp.
COLEO	**Haliplidae**	Haliplus sp.	Haliplus
COLEO	**Dryopidae**	Helichus sp.	Helichus
COLEO	**Elmidae**	Heterlimnius sp.	Heterlimnius
COLEO	**Dytiscidae**	Liodessus sp.	Liodessus
COLEO	**Elmidae**	Narpus sp.	Narpus
COLEO	**Elmidae**	Optioservus sp.	Optioservus
COLEO	**Dytiscidae**	Oreodytes sp.	Oreodytes
COLEO	**Elmidae**	Zaitzevia sp.	Zaitzevia
MEGALO	**Sialidae**	Sialis sp.	Sialis
DIPTERA	**Chironomidae**	Ablabesmyia sp.	Chironomidae*
DIPTERA	**Chironomidae**	Boreoheptagyia sp.	Chironomidae*
DIPTERA	**Chironomidae**	Brillia sp.	Chironomidae*
DIPTERA	**Chironomidae**	Cardiocladius sp.	Chironomidae*
DIPTERA	**Chironomidae**	Chaetocladius sp.	Chironomidae*

DIPTERA	**Chironomidae**	Cladotanytarsus sp.	Chironomidae*
DIPTERA	**Chironomidae**	Corynoneura sp.	Chironomidae*
DIPTERA	**Chironomidae**	Cricotopus (Nostoc.) nostocicola	Chironomidae*
DIPTERA	**Chironomidae**	Cricotopus bicinctus gr.	Chironomidae*
DIPTERA	**Chironomidae**	Cricotopus sp.	Chironomidae*
DIPTERA	**Chironomidae**	Cricotopus trifascia gr.	Chironomidae*
DIPTERA	**Chironomidae**	Cryptochironomus sp.	Chironomidae*
DIPTERA	**Chironomidae**	Diamesa sp.	Chironomidae*
DIPTERA	**Chironomidae**	Eukiefferiella brehmi gr.	Chironomidae*
DIPTERA	**Chironomidae**	Eukiefferiella claripennis gr.	Chironomidae*
DIPTERA	**Chironomidae**	Eukiefferiella coerulescens gr.	Chironomidae*
DIPTERA	**Chironomidae**	Eukiefferiella devonica gr.	Chironomidae*
DIPTERA	**Chironomidae**	Eukiefferiella gracei gr.	Chironomidae*
DIPTERA	**Chironomidae**	Eukiefferiella sp.	Chironomidae*
DIPTERA	**Chironomidae**	Euryhapsis sp.	Chironomidae*
DIPTERA	**Chironomidae**	Heleniella sp.	Chironomidae*
DIPTERA	**Chironomidae**	Hydrobaenus sp.	Chironomidae*

DIPTERA	**Chironomidae**	Lopescladius sp.	Chironomidae*
DIPTERA	**Chironomidae**	Micropsectra sp.	Chironomidae*
DIPTERA	**Chironomidae**	Micropsectra/Tanytarsus sp.	Chironomidae*
DIPTERA	**Chironomidae**	Microtendipes pedellus gr.	Chironomidae*
DIPTERA	**Chironomidae**	Nanocladius sp.	Chironomidae*
DIPTERA	**Chironomidae**	Odontomesa sp.	Chironomidae*
DIPTERA	**Chironomidae**	Orthocladius (Euortho.) rivulorum	Chironomidae*
DIPTERA	**Chironomidae**	Orthocladius (Euorthocladius) sp.	Chironomidae*
DIPTERA	**Chironomidae**	Orthocladius Complex	Chironomidae*
DIPTERA	**Chironomidae**	Orthocladius sp.	Chironomidae*
DIPTERA	**Chironomidae**	Pagastia sp.	Chironomidae*
DIPTERA	**Chironomidae**	Parakiefferiella sp.	Chironomidae*
DIPTERA	**Chironomidae**	Paralauterborniella nigrohalteralis	Chironomidae*
DIPTERA	**Chironomidae**	Parametriocnemus sp.	Chironomidae*
DIPTERA	**Chironomidae**	Paratanytarsus sp.	Chironomidae*
DIPTERA	**Chironomidae**	Pentaneura sp.	Chironomidae*

DIPTERA	**Chironomidae**	Pentaneurini	Chironomidae*
DIPTERA	**Chironomidae**	Phaenopsectra sp.	Chironomidae*
DIPTERA	**Chironomidae**	Polypedilum sp.	Chironomidae*
DIPTERA	**Chironomidae**	Potthastia gaedii gr.	Chironomidae*
DIPTERA	**Chironomidae**	Potthastia longimana gr.	Chironomidae*
DIPTERA	**Chironomidae**	Procladius sp.	Chironomidae*
DIPTERA	**Chironomidae**	Pseudochironomus sp.	Chironomidae*
DIPTERA	**Chironomidae**	Rheocricotopus sp.	Chironomidae*
DIPTERA	**Chironomidae**	Rheosmittia sp.	Chironomidae*
DIPTERA	**Chironomidae**	Rheotanytarsus sp.	Chironomidae*
DIPTERA	**Chironomidae**	Stempellina sp.	Chironomidae*
DIPTERA	**Chironomidae**	Stempellinella sp.	Chironomidae*
DIPTERA	**Chironomidae**	Stictochironomus sp.	Chironomidae*
DIPTERA	**Chironomidae**	Sublettea sp.	Chironomidae*
DIPTERA	**Chironomidae**	Synorthocladius sp.	Chironomidae*
DIPTERA	**Chironomidae**	Tanytarsus sp.	Chironomidae*
DIPTERA	**Chironomidae**	Thienemanniella sp.	Chironomidae*
DIPTERA	**Chironomidae**	Thienemannimyia gr. sp.	Chironomidae*

DIPTERA	**Chironomidae**	Tribelos jucundum	Chironomidae*
DIPTERA	**Chironomidae**	Tvetenia bavarica gr.	Chironomidae*
DIPTERA	**Chironomidae**	Tvetenia discoloripes gr.	Chironomidae*
DIPTERA	**Tipulidae**	Antocha sp.	Antocha
DIPTERA	**Athericidae**	Atherix sp.	Atherix
DIPTERA	**Ceratopogonidae**	Bezzia/Palpomyia sp.	Bezzia/Palpomyia sp.
DIPTERA	**Stratiomyidae**	Caloparyphus sp.	Caloparyphus sp.
DIPTERA	**Ceratopogonidae**	Ceratopogoninae	Ceratopogoninae
DIPTERA	**Empididae**	Chelifera/Metachela sp.	Chelifera/Metachela sp.
DIPTERA	**Empididae**	Clinocera sp.	Clinocera sp.
DIPTERA	**Tipulidae**	Cryptolabis sp.	Cryptolabis sp.
DIPTERA	**Ceratopogonidae**	Dasyhelea sp.	Dasyhelea sp.
DIPTERA	**Tipulidae**	Dicranota sp.	Dicranota sp.
DIPTERA	**Empididae**	Hemerodromia sp.	Hemerodromia sp.
DIPTERA	**Tipulidae**	Hexatoma sp.	Hexatoma sp.
DIPTERA	**Tipulidae**	Limnophila sp.	Limnophila sp.
DIPTERA	**Muscidae**	Muscidae	Muscidae

DIPTERA	**Empididae**	Neoplasta sp.	Neoplasta sp.
DIPTERA	**Tipulidae**	Pedicia sp.	Pedicia sp.
DIPTERA	**Psycodidae**	Pericoma/Telmatoscopus sp.	Pericoma
DIPTERA	**Ceratopogonidae**	Probezzia sp.	Probezzia
DIPTERA	**Tipulidae**	Rhabdomastix fascigera gr.	Rhabdomastix
DIPTERA	**Sciomyzidae**	Sciomyzidae	Sciomyzidae
DIPTERA	**Simuliidae**	Simulium sp.	Simulium
DIPTERA	**Tipulidae**	Tipula sp.	Tipula
DIPTERA	**Empididae**	Wiedemannia sp.	Wiedemannia
TRICHOP	**Brachycentridae**	Amiocentrus aspilus	Amiocentrus
TRICHOP	**Apatanidae**	Apatania sp.	Apatania
TRICHOP	**Arctopsychidae**	Arctopsyche grandis	Arctopsyche grandis
TRICHOP	**Brachycentridae**	Brachycentridae	Brachycentridae
TRICHOP	**Brachycentridae**	Brachycentrus americanus	Brachycentrus
TRICHOP	**Brachycentridae**	Brachycentrus occidentalis	Brachycentrus
TRICHOP	**Leptoceridae**	Ceraclea sp.	Ceraclea
TRICHOP	**Hydropsychidae**	Cheumatopsyche sp.	Cheumatopsyche
TRICHOP	**Glossomatidae**	Culoptila sp.	Culoptila

TRICHOP	**Limnephilidae**	Dicosmoecus gilvipes	Dicosmoecus gilvipes
TRICHOP	**Philopotamidae**	Dolophilodes sp.	Dolophilodes
TRICHOP	**Glossomatidae**	Glossosoma sp.	Glossosoma
TRICHOP	**Helicopsychidae**	Helicopsyche sp.	Helicopsyche
TRICHOP	**Hydropsychidae**	Hydropsyche sp.	Hydropsyche
TRICHOP	**Hydroptilidae**	Hydroptila sp.	Hydroptila
TRICHOP	**Hydroptilidae**	Hydroptilidae	Hydroptilidae
TRICHOP	**Lepidosomatidae**	Lepidostoma sp.	Lepidostoma
TRICHOP	**Brachycentridae**	Micrasema sp.	Micrasema
TRICHOP	**Leptoceridae**	Nectopsyche sp.	Nectopsyche
TRICHOP	**Limnephilidae**	Neothremma sp.	Neothremma
TRICHOP	**Hydroptilidae**	Neotrichia sp.	Neotrichia
TRICHOP	**Hydroptilidae**	Ochrotrichia sp.	Ochrotrichia
TRICHOP	**Leptoceridae**	Oecetis avara	Oecetis
TRICHOP	**Leptoceridae**	Oecetis disjuncta	Oecetis
TRICHOP	**Leptoceridae**	Oecetis sp.	Oecetis
TRICHOP	**Limnephilidae**	Oligophlebodes sp.	Oligophlebodes
TRICHOP	**Hydroptilidae**	Oxyethira sp.	Oxyethira

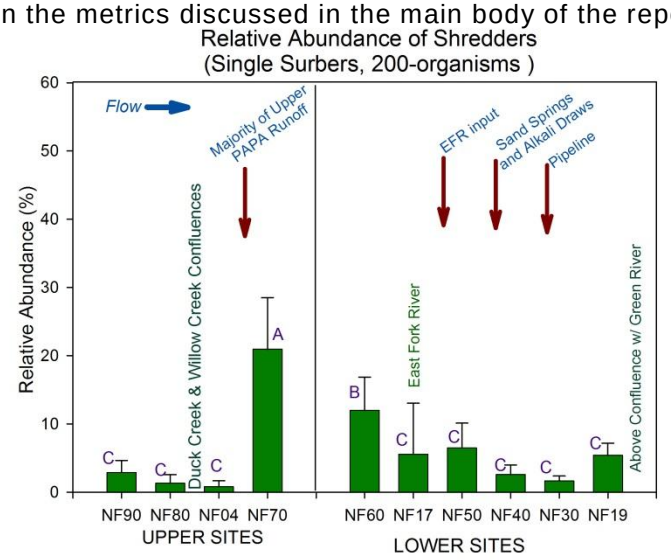
TRICHOP	**Hydropsychidae**	Parapsyche elsis	Parapsyche elsis
TRICHOP	**Glossomatidae**	Protoptila sp.	Protoptila
TRICHOP	**Rhyacophilidae**	Rhyacophila brunnea gr.	Rhyacophila
TRICHOP	**Rhyacophilidae**	Rhyacophila coloradensis gr.	Rhyacophila
TRICHOP	**Rhyacophilidae**	Rhyacophila hyalinata gr.	Rhyacophila
TRICHOP	**Rhyacophilidae**	Rhyacophila pellisa/valuma	Rhyacophila
TRICHOP	**Rhyacophilidae**	Rhyacophila sp.	Rhyacophila
TRICHOP	**Rhyacophilidae**	Rhyacophila vofixa gr.	Rhyacophila
LEPIDOP	**Pyralidae**	Petrophila sp.	Petrophila
NONI	**Gastropoda**	Ferrissia sp.	Ferrissia
NONI	**Gastropoda**	Fluminicola sp.	Fluminicola
NONI	**Gastropoda**	Fossaria sp.	Fossaria
NONI	**Gastropoda**	Gyraulus sp.	Gyraulus
NONI	**Gastropoda**	Lymnaeidae	Lymnaeide
NONI	**Gastropoda**	Physa sp.	Physa
NONI	**Gastropoda**	Planorbidae	Planorbidae
NONI	**Gastropoda**	Valvata lewisi	Valvata lewisi
NONI	**Bivalva**	Pisidium sp.	Sphaeriidae

NONI	**Bivalva**	Sphaeriidae	Sphaeriidae
NONI	**Bivalva**	Sphaerium sp.	Sphaeriidae
NONI	**Bivalva**	Unionacea	Unionacea
NONI	**Oligo**	Chaetogaster diastrophus	Chaetogaster
NONI	**Oligo**	Enchytraeidae	Enchytraeidae
NONI	**Oligo**	Erpobdellidae	Erpobdellidae
NONI	**Hirudinea**	Glossiphonia complanata	Glossiphonia
NONI	**Oligo**	Haplotaxis sp.	Haplotaxis
NONI	**Hirudinea**	Helobdella stagnalis	Helobdella stagnalis
NONI	**Oligo**	Lumbricina	Lumbricina
NONI	**Oligo**	Lumbriculidae	Lumbriculidae
NONI	**Oligo**	Nais behningi	Nais
NONI	**Oligo**	Nais bretscheri	Nais
NONI	**Oligo**	Nais sp.	Nais
NONI	**Oligo**	Ophidonais serpentina	Ophidonais serpentina
NONI	**Oligo**	Slavina appendiculata	Slavina appendiculata
NONI	**Oligo**	Tubificidae w/ cap setae	Naididae (Tubificidae)
NONI	**Oligo**	Tubificidae w/o cap setae	Naididae (Tubificidae)

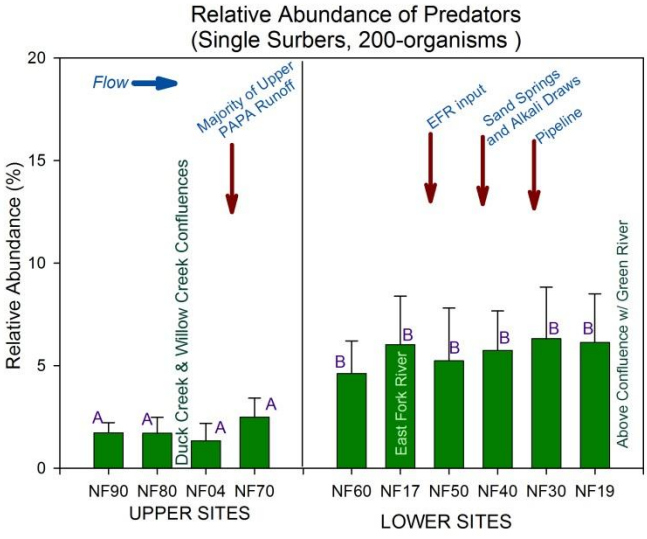
NONI	**Acari**	Acari	Acari
NONI	**Acari**	Atractides sp.	Acari
NONI	**Acari**	Aturus sp.	Acari
NONI	**Acari**	Hygrobates sp.	Acari
NONI	**Acari**	Lebertia sp.	Acari
NONI	**Acari**	Oribatei	Acari
NONI	**Acari**	Protzia sp.	Acari
NONI	**Acari**	Sperchon sp.	Acari
NONI	**Acari**	Sperchonopsis sp.	Acari
NONI	**Acari**	Torrenticola sp.	Acari
NONI	**Amphipoda**	Gammarus sp.	Gammarus sp.
NONI	**Amphipoda**	Hyalella sp.	Hyalella sp.
NONI	**Nematoda**	Nematoda	Nematoda
NONI	**Turbellaria**	Polycelis sp.	Polycelis sp.
	Turbellaria	Turbellaria	Turbellaria

*Rabeni and Wang. 2001. Bioassessment of streams using macroinvertebrates: are Chironomidae necessary? *Environmental Monitoring and Assessment* 71: 177–185, 2001

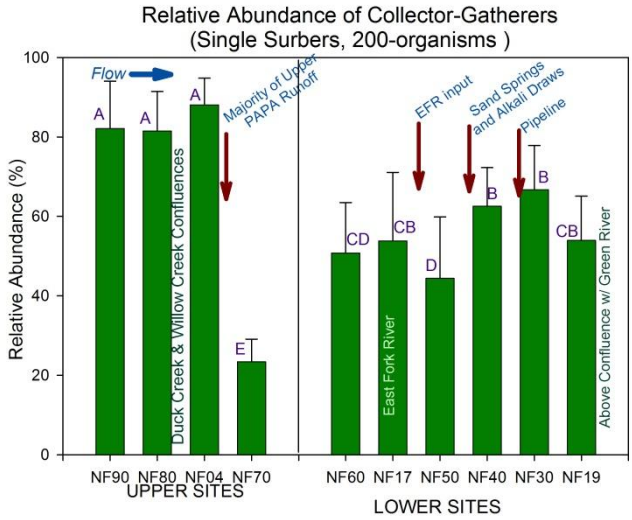
APPENDIX 2: Supplemental Graphs. The relative abundance of different functional feeding groups is wrought with problems of non-independence of observation and multicolinearity. For this reason we decided, *a priori*, to examine collectors and scrapers. Still, for diagnostic purposes, it is useful to consider which groups went up when others went down. For this reason, we added these graphs to enable interested readers to infer the types of changes that accompanied changes in the metrics discussed in the main body of the report.



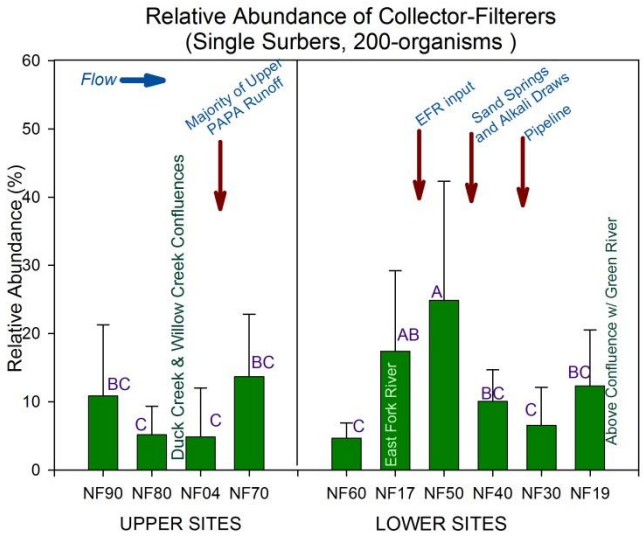
Appendix Figure 2.1. Percent Shredders. The percent contribution of shredders to benthic communities of the New Fork River in 2010. Error bars are 95% confidence intervals.



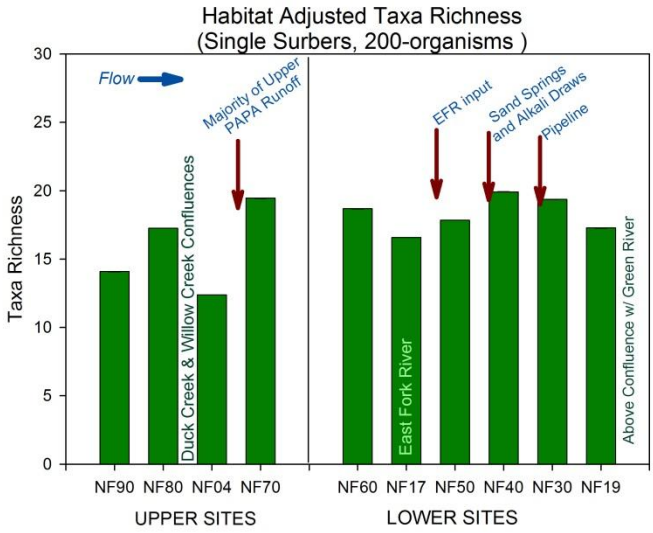
Appendix Figure 2.2 Percent Predators. The percent contribution of predators to benthic communities of the New Fork River in 2010. Error bars are 95% confidence intervals.



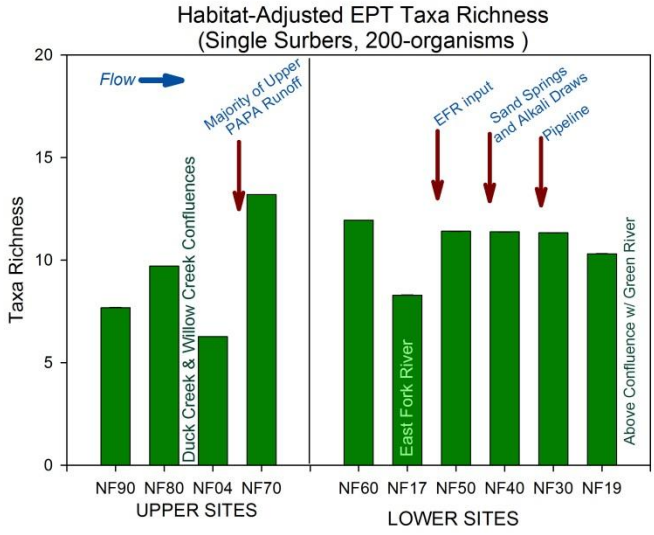
Appendix Figure 2.3. Percent Collector-Gatherers. The percent contribution of collector-gatherers to benthic communities of the New Fork River in 2010. Error bars are 95% confidence intervals.



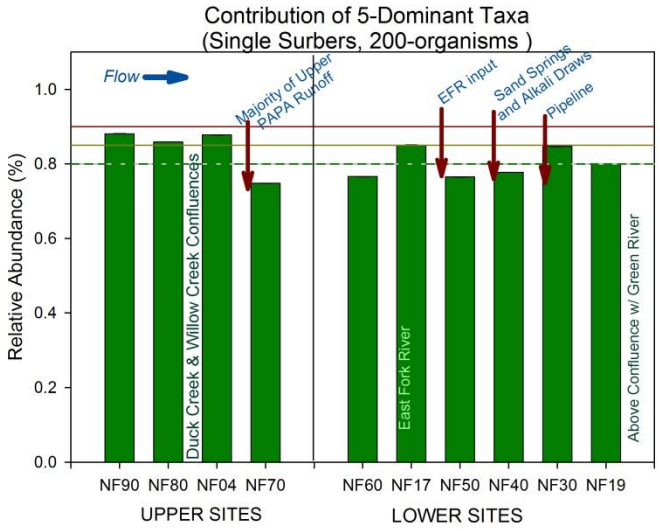
Appendix Figure 2.4. Percent Collector-Filterers. The percent contribution of collector-filterers to benthic communities of the New Fork River in 2010. Error bars are 95% confidence intervals.



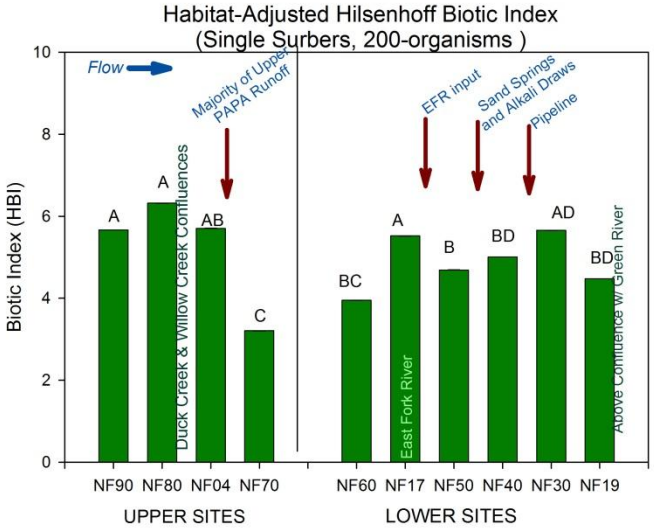
Appendix Figure 2.4. Taxa Richness. The Graph shows the habitat-adjusted EPT richness of benthic communities of the New Fork River in 2010. There are no error-bars because data are results of a modeling procedure used to estimate the site-specific covariate-adjusted means.



Appendix Figure 2.5. Adjusted EPT Richness. The Graph shows the habitat-adjusted EPT richness of benthic communities of the New Fork River in 2010. There are no error-bars because data are results of a modeling procedure used to estimate the site-specific covariate-adjusted means.



Appendix Figure 2.6. Dominance (5 Taxa). The Graph shows the habitat-adjusted Dominance of five abundant taxa among benthic communities of the New Fork River in 2010. There are no error-bars because data are results of a modeling procedure used to estimate the site-specific covariate-adjusted means. Colored references show values less than 80% are desired, whereas values greater than 90% are not.



Appendix Figure 2.7. Adjusted HBI. The Graph shows the habitat-adjusted Hilsenhoff Biotic Index for benthic communities of the New Fork River in 2010. There are no error-bars because data are results of a modeling procedure used to estimate the site-specific covariate-adjusted means.

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